

**MATING STRATEGIES AND SPERM COMPETITION
IN NEW ZEALAND GECKOS
(FAMILY GEKKONIDAE)**

A thesis
submitted in fulfilment
of the requirements for the Degree
of
Doctor of Philosophy in Zoology
in the
University of Canterbury
by
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University of Canterbury

2003

Contents

Abstract	1
Chapter 1: General Introduction	2
Taxonomy of New Zealand Geckos	6
Chapter 2: Testis Size as a Predictor of Mating System	7
Introduction	7
Methods	10
Results	14
Discussion	22
Conclusions	26
Chapter 3: Male Reproductive Characteristics and Sperm Competition	27
Introduction	27
Methods	31
Results	41
Discussion	48
Conclusions	51
Chapter 4: Female Reproductive Morphology and Sperm Competition	52
Introduction	52
Methods	55
Results	58
Discussion	67
Conclusions	72

Chapter 5: The Social Mating System of <i>Hoplodactylus maculatus</i>	73
Introduction	73
Methods	75
Results	82
Discussion	94
Conclusions	98
Chapter 6: Sperm Morphology of <i>Hoplodactylus maculatus</i>	99
Introduction	99
Methods	100
Results	102
Discussion	107
Chapter 7: Effect of Temperature on Sperm Motility in <i>Hoplodactylus maculatus</i>	111
Introduction	111
Methods	113
Results	116
Discussion	121
Conclusions	125
Chapter 8: General Discussion	126
Future Research	131
Acknowledgements	134
References	136
Appendix A	175
Appendix B	182

List of Figures

Figure	Page
2.1 Relationship between left and right testis volume in New Zealand geckos	14
2.2 Variation in residual testis volume between months for <i>Hoplodactylus maculatus</i> from Turakirae Head	15
2.3 Relationship between mean testis volume and SVL in New Zealand geckos	16
2.4 Relationship between mean testis volume and SVL across species of lizards and snakes	16
2.5 Relative testis size of lizards and snakes	18
3.1 Diagram of inverted hemipenis showing the location of measurements _	32
3.2 SEM micrographs of hemipenes of <i>Hoplodactylus maculatus</i> “Canterbury”: A. Covered with mucus; B. With mucus removed _	35
3.3 SEM micrographs of hemipenes of <i>Hoplodactylus maculatus</i> prepared using: A. Potassium hydroxide - good condition; B. No treatment; C. Potassium hydroxide - damaged; D. Saponin	37
3.4 Relationship between everted hemipenis length and mean testis volume residual in New Zealand geckos	42
3.5 Relationship between residual testis volume, hemipenis size and season in New Zealand geckos: A. Length; B. Width	43
3.6 SEM micrographs of hemipenis of <i>Hoplodactylus maculatus</i> : A. Sulcal surface; B. Asulcal surface	45
3.7 SEM micrograph of calyces covering hemipenis showing the location of measurements	45
3.8 Sperm from two New Zealand gecko species: A. <i>Hoplodactylus pacificus</i> ; B. <i>H. maculatus</i> “Canterbury”	46
3.9 Relationship between mean testis volume residual and sperm length in New Zealand geckos	48
4.1 Longitudinal dissection of oviduct of <i>Naultinus elegans punctatus</i> . . .	56

4.2	SSTs in the uterine tube of <i>Hoplodactylus maculatus</i> :	
	A. Whole-mount; B. Histological section	59
4.3	Transverse section of the vagina of <i>Hoplodactylus maculatus</i>	60
4.4	Relationship between number of folds in the vagina and mean relative testis volume in New Zealand geckos during:	
	A. Sperm storage season; B. Non-sperm storage season	65
5.1	Study site at Devil's Knob, Banks Peninsula:	
	A. Location map; B. Photograph	76
5.2	<i>Hoplodactylus maculatus</i>	77
5.3	Diagram of enclosure design during: A. 2001; B. 2002	80
5.4	Mean number of <i>Hoplodactylus maculatus</i> captured across months	82
5.5	Size distribution of <i>Hoplodactylus maculatus</i>	84
5.6	Mean temperature during 2001 and 2002: A. Maximum; B. Minimum _	85
5.7	Mean number of courtships/h across months for <i>Hoplodactylus maculatus</i>	86
5.8	Relationship between SVL size rank and mean courtship rate in male <i>Hoplodactylus maculatus</i> during: A. 2001; B. 2002	88
5.9	Relationship between total body length size rank and mean courtship rate in male <i>Hoplodactylus maculatus</i> during: A. 2001; B. 2002	88
5.10	Relationship between total body length size rank and mean courtship rate in female <i>Hoplodactylus maculatus</i> during: A. 2001; B. 2002	89
5.11	Temporal variation in mean fight rate of <i>Hoplodactylus maculatus</i> during: A. 2001; B. 2002	91
5.12	Relationship between time of day and mean fight rate in <i>Hoplodactylus</i> <i>maculatus</i> across months	92
5.13	Relationship between size rank difference and mean attack rate in <i>Hoplodactylus maculatus</i>	93
6.1	SEM micrographs of two sperm morphs from <i>Hoplodactylus maculatus</i> : A. Long; B. Short	102
6.2	Light micrographs of long and short sperm morphs from <i>Hoplodactylus maculatus</i>	103

6.3	Two sperm morphs from <i>Hoplodactylus maculatus</i> stained with Hoescht 33342: A. Long; B. Short	105
6.4	Two sperm morphs from <i>Hoplodactylus maculatus</i> stained with ethidium bromide: A. Long; B. Short	106
7.1	Relationship between sperm tail beat frequency and time in samples from <i>Hoplodactylus maculatus</i>	117
7.2	Mean proportion of motile short sperm in samples from <i>Hoplodactylus</i> <i>maculatus</i> at three temperatures	118
7.3	Relationship between sample number and mean proportion of motile short sperm in samples from <i>Hoplodactylus maculatus</i>	118
7.4	Mean tail beat frequency of short sperm in samples from <i>Hoplodactylus</i> <i>maculatus</i> at three temperatures	119
7.5	Effect of time on short sperm motility at three temperatures in samples from <i>Hoplodactylus maculatus</i>	120

List of Tables

Table	Page
2.1 Sex differences in SVL for New Zealand geckos	19
2.2 Sex differences in head dimensions for New Zealand geckos	20
4.1 Intraspecific variation in number of folds and number and size of SSTs in regions of the oviduct in <i>Hoplodactylus maculatus</i>	61
4.2 Interspecific variation in number of folds and number and size of SSTs in regions of the oviduct in New Zealand geckos	63
4.3 Relationship between number of folds and number and size of SSTs in the oviduct, season and mean testis volume residual	64
4.4 Relationship between number of folds and number and size of SSTs in the oviduct, season and sperm length	66
7.1 Effect of temperature, sample number and time on the proportion of motile short sperm in samples from <i>Hoplodactylus maculatus</i>	118
7.2 Effect of temperature, sample number and time on tail beat frequency of short sperm in samples from <i>Hoplodactylus maculatus</i>	119

Abstract

Most species of reptile studied to date have polygynandrous mating systems and possess specialised sperm storage regions. Consequently, there is a high potential for sperm competition in this group. Using comparative analyses, I examined the level of sperm competition in New Zealand geckos and how this has influenced the evolution of their reproductive morphology. Across lizards and snakes, there was more than a 40-fold variation in relative testis size. New Zealand geckos fell in the middle of this range and lacked sexual dimorphism in head size, suggesting that most species have polygynandrous mating systems. I confirmed this for one species, *Hoplodactylus maculatus*, which is gregarious, lacks territoriality and has a courtship pattern that suggests a high level of promiscuity for both sexes. I found that hemipenis size in New Zealand geckos was positively correlated with relative testis size, suggesting that sperm competition has resulted in the evolution of larger intromittent organs. However, the surface features of the hemipenis were relatively conservative across species. Although there was no relationship between sperm length or putative sperm storage site (SST) morphology and relative testis size, species with fewer SSTs, and thus more intense sperm competition, had longer sperm. *H. maculatus* males produced two types of sperm which differed not only in length but also in fertilising capacity, the short morph lacking DNA. This is the first known example of such sperm polymorphism in a vertebrate and may have evolved in response to sperm competition, the non-fertilising morph potentially helping to block the sperm of rival males or filling sperm storage sites. The motility of these short sperm was positively correlated with temperature; however, at higher temperatures motility declined with time, suggesting a trade-off between motility and longevity. Such temperature influences on male reproductive physiology have important implications for males of ectothermic species under sperm competition.

Chapter 1: General Introduction

The theory of sperm competition, defined as competition between the sperm of two or more males for fertilisation of a female's ova, was first proposed by Parker (1970). In order for sperm competition to be possible, there must be temporal overlap of sperm from more than one male within a female's reproductive tract; thus females must mate with and store the sperm of several males before her ova are fertilised (Parker, 1970). Parker (1970) believed that these prerequisites for sperm competition were best met in the insects, as most insect species possess sperm storage organs and their sperm are often very long-lived. However, studies have shown that sperm competition is a widespread phenomenon which can have significant evolutionary consequences for both male and female reproductive morphology across a wide range of taxa (for reviews see Birkhead, 1987; Birkhead and Hunter, 1990; Birkhead and Møller, 1998; Ginsberg and Huck, 1989) and can even occur in species with external fertilisation (e.g. fish [Balshine et al., 2001; Petersen and Warner, 1998; Stockley et al., 1997] and amphibians [Halliday, 1998; Jennions and Passmore, 1993; Kusano et al., 1991]). One group that has largely been overlooked in studies investigating the relationship between sperm competition and reproductive morphology is the reptiles (Class Reptilia).

Although much information exists regarding the taxonomy, general ecology and reproductive cycles of reptiles, it is only over the last 20 years that questions relating to mating behaviour, reproductive success and sperm competition have been addressed (see reviews by Devine, 1984; Olsson and Madsen, 1998). Moreover, relatively few species have been studied in depth, so that there is a paucity of basic information available concerning the mating system and reproductive morphology of reptiles. This makes comparative studies addressing broad evolutionary questions particularly difficult. Despite this, information currently coming to light indicates that the Reptilia are likely to present an interesting study group when addressing evolutionary questions implicating sperm competition.

Recent evidence suggests that the requirements for sperm competition are met in the majority of reptiles. Comparative studies have shown that most species are probably polygynandrous (Devine, 1984; Olsson and Madsen, 1998), that is both males and females mate promiscuously. Moreover, many reptilian species possess sperm storage

sites (Girling, 2002; Gist and Jones, 1987; Olsson and Madsen, 1998; Sever and Hamlett, 2002) and reptiles are capable of storing sperm for much longer periods than any other vertebrate group (Birkhead and Møller, 1993; Devine, 1984). In addition, in some species mechanisms have been identified that are believed to reduce the incidence of female remating and thus sperm competition, such as the formation of copulatory plugs (Devine, 1975, 1977; Ross and Crews, 1977; Shine et al., 2000c) and the production of pheromones in the seminal fluid (Shine et al., 2000c). Therefore it is probable that sperm competition has played a significant role in the evolution of mating strategies and reproductive morphology in reptiles; moreover, it is even possible that sperm competition is more intense in this group than in any other vertebrate group studied to date. Consequently, studies addressing evolutionary hypotheses concerning reproductive strategies and sperm competition in this group are likely to be very rewarding.

New Zealand geckos (Family Gekkonidae) are an excellent model group for comparative studies investigating the role of sperm competition in the evolution of reproductive morphology and behaviour, due to the large number of very closely related species inhabiting a relatively narrow geographical range. There are two genera of geckos in New Zealand, *Hoplodactylus* Fitzinger and *Naultinus* Gray. These differ widely in their habits, with *Hoplodactylus* spp. being nocturnal, terrestrial, ‘brown’ geckos, whereas *Naultinus* spp. are diurnal, arboreal, ‘green’ geckos. Consequently, it is expected that the reproductive characteristics of these two genera may differ greatly. To date, research on members of this group has primarily focussed on taxonomy and general ecology (e.g. Daugherty et al., 1994; Hitchmough, 1997; McIvor, 1972; Thompson et al., 1992; Whitaker, 1968, 1982). However, several studies have investigated their reproductive physiology in greater detail.

The reproductive ecology of New Zealand geckos is of particular interest due to the high incidence of viviparity, which is rare amongst gekkonid lizards, only being found in one other species living in New Caledonia (Henkel and Schmidt, 1995). Consequently, a lot of research in New Zealand has focussed on the mechanisms of placentation and reproductive physiology of female geckos (Girling et al., 1997, 1998). In addition, the reproductive cycles of both sexes have been investigated for several species (Cree, 1994; Cree and Guillette, 1995; Cree et al., 2003; Fawcett, 1972; MacAvoy, 1976; McIvor, 1972; Robinson, 1985; Towns, 1975; Wilson and Cree, 2003). These

investigations have shown that sperm storage sites are present in the female oviduct of several species (Girling et al., 1997, 1998; MacAvoy 1976). Moreover, in many species male and female reproductive cycles are asynchronous, so that copulation occurs several months prior to ovulation (MacAvoy, 1976). This means that there is a period of obligatory sperm storage and that the prerequisites for sperm competition are met in this group. However, no information is available concerning the mating system of any species and no studies to date have investigated the relationship between mating system and reproductive morphology in New Zealand lizards.

In the first section of this study I use the comparative method to make predictions about the mating system of New Zealand gecko species and to investigate evolutionary forces that may have contributed to variation in both male and female reproductive morphology. Across many taxa there is a strong correlation between relative testis size and mating system (e.g. Gage, 1994; Harcourt et al., 1981; Kusano et al., 1991; Møller, 1991; Short, 1979; Stockley et al., 1997). Since no information is available concerning the mating system of any species of New Zealand gecko, in Chapter 2 I compare relative testis size of New Zealand geckos with that of lizards belonging to several families found elsewhere in the world. I use this information to extrapolate the likely mating system of each New Zealand species.

Sexual selection can strongly affect male reproductive morphology. In particular, males belonging to species with intense sperm competition often evolve elaborate intromittent organs that are capable of displacing or removing the sperm of rival males, or stimulating the female to better position their sperm within her reproductive tract (Brownell and Ralls, 1986; Coker et al., 2002; Dixon, 1987; Eberhard, 1985; Gage, 1992; Harcourt and Gardiner, 1994). The mating system of a species can also affect sperm characteristics, resulting in an increase in either sperm number (Birkhead et al., 1993; Briskie, 1993; Evans et al., 2003; Gage and Barnard, 1996; Stockley et al., 1997; Svärd and Wiklund, 1989) or size (Balshine et al., 2001; Briskie et al., 1997; Dixon and Birkhead, 1997; Gage, 1994; Gomendio and Roldan, 1991; LaMunyon and Ward, 2002; Morrow and Gage, 2000; Pitnick and Markow, 1994). Therefore, in Chapter 3 I investigate the degree of variation in intromittent organ morphology and ejaculate characteristics of male New Zealand geckos and determine whether this variation is related to differences in mating systems across species.

In birds, sperm storage site morphology is often correlated with mating system (Briskie and Montgomerie, 1993; Briskie et al., 1997), with females belonging to species with more intense sperm competition having less numerous and/or larger storage areas. As females of several species of New Zealand gecko have sperm storage sites in their oviducts (Girling et al., 1997, 1998; MacAvoy 1976), in Chapter 4 I investigate variation in sperm storage site morphology in New Zealand geckos and relate this to mating system.

In the second part of this study, I investigate the mating strategies and male reproductive morphology in one species of New Zealand gecko, *Hoplodactylus maculatus*, a species known to have sperm storage sites and a period of obligatory sperm storage (Girling et al., 1997, 1998; MacAvoy, 1976). The mating system of *H. maculatus* is determined from a combination of behavioural observations of captive populations and estimations of population parameters from a field population (Chapter 5). This information enables me to determine the accuracy of predictions made in Chapter 2 regarding the mating system of this species.

In Chapter 6, I examine the sperm characteristics of *H. maculatus* in more detail. I found that males of this species produce two types of sperm: long fertilising and short non-fertilising. These closely resemble those observed in Lepidoptera (Silberglied et al., 1984; Swallow and Wilkinson, 2002). This is the first known example of sperm polymorphism in vertebrates. I describe these two sperm morphs and discuss their possible functions.

In externally fertilising species, temperature can have strong effects on sperm swimming speeds and thus fertilisation success (Billard and Cosson, 1992; Levitan, 2000). However, no such effect has ever been investigated in reptiles, despite sperm being stored and transferred at variable temperatures, which are often sub-optimal in nocturnal species such as *H. maculatus*. To determine what role temperature might play in the outcome of sperm competition, in the final chapter (Chapter 7) I investigate the effect of temperature on sperm motility.

The use of multiple techniques to determine the mating strategies and reproductive traits of New Zealand geckos enables me to draw some broad conclusions concerning the evolution of reproductive characteristics despite the paucity of knowledge generally available for these species. Although in many cases I must infer mating system through indirect measures, my conclusions are supported by the findings for one species,

H. maculatus, which I have investigated in much greater detail. Together, my results indicate that sperm competition has played a pivotal role in the evolution of mating behaviour and reproductive physiology of New Zealand geckos.

Taxonomy of New Zealand Geckos

There has been great interest in the taxonomy of New Zealand geckos, resulting in a certain degree of confusion concerning the taxonomic status of some species. Consequently, several points require clarification.

Firstly, it should be noted that members of the genus *Heteropholis* Fischer have now been placed in *Naultinus* Gray (first suggested by Thomas (1982); formalised by Bauer (1990, not seen but cited by Whitaker AH, pers. comm.)). *Hoplodactylus maculatus* Boulenger was synonymised with *Hoplodactylus pacificus* Gray by McCann (1955). However, Robb and Rowlands (1977) have since resurrected *H. maculatus* and redescribed both species. Therefore, it is now believed that *H. pacificus* is restricted to the North Island and offshore islands, and all previous references to this species from the South Island are now considered to be *H. maculatus*. In addition, the status of *H. maculatus* is currently being revised, as it is believed to represent a species complex (Hitchmough, 1991, abstract; Hitchmough, 1997). Consequently, since each potentially new species originates from a distinct geographic location, members of this complex are given specific status in comparative analyses and are referred to as *H. maculatus* “locality”. Finally, Robb and Hitchmough (1980) identified two subspecies of *Naultinus elegans* Gray (*N. e. elegans* and *N. e. punctatus*). Since these subspecies have very little geographic overlap and differ greatly in size (Robb and Hitchmough, 1980) I have treated these as if they were separate species in all analyses.

Chapter 2: Testis Size as a Predictor of Mating System

Introduction

In vertebrates, the testes have the dual function of producing both hormones and sperm. Consequently, as the body size of an individual increases, so too does the requirement, and thus size, of the testes (Gage, 1994; Harcourt et al., 1981; Kenagy and Trombulak, 1986). However, in addition to this size related growth of testes, it has also been found that across a wide range of taxa there is often a large degree of variation in relative testis size (that is, the size of testes after accounting for variation in body size) between closely related species (e.g. mammals [Harcourt et al., 1981; Heske and Ostfeld, 1990; Møller, 1989; Short, 1979], birds [Møller, 1988, 1991], amphibians [Emerson, 1997; Kusano et al., 1991] and reptiles [Olsson and Madsen, 1998]). One factor which may contribute to this size variation is the hormonal requirements of a species. Males belonging to species where dominance hierarchies play an important role in the social system may need to produce more hormones and thus require larger relative testis size, as shown to be the case in capybaras (Moreira et al., 1997). However, in a study on frogs, Emerson (1997) showed that although there was a positive correlation between testis size and the level of androgens produced, this did not translate into variation in the degree of agonistic behaviour. Moreover, many species of invertebrates also show a similarly great variation in relative testis size (Gage, 1994); since invertebrate testes do not function to produce hormones (Gillott, 1980) further explanations for this trend are required.

Testis size is strongly correlated with sperm production in several taxa (Breed, 1997; Harcourt et al., 1981; Harvey and Harcourt, 1984; Møller, 1988, 1989). Therefore, it is possible that variation in relative testis size is due to males of a species needing to produce different numbers or concentrations of sperm. Several factors have been recognised as possible contributors to this requirement. For example, the geographic range of a species, or latitude at which it lives, may be expected to affect the demand upon sperm supply: species that live at higher latitudes often have shorter breeding seasons than those in more tropical climates; this should result in males of temperate species mating more frequently over a shorter time period, and thus requiring larger

testes. However, although several studies have investigated the relationship between testis size, latitude and seasonality of breeding, results to date are inconclusive, and often show no correlation between these variables (Harcourt et al., 1995; Hosken, 1998; Kusano et al., 1991; Pitcher and Stutchbury, 1998; Ribble and Millar, 1992). The density of animals in a population could similarly affect the sperm production demands upon a male and thus testis size: in birds it has been shown that males living in regions with high nest densities have larger relative testis size than those living in areas of lower density (Møller, 1991). However, the opposite pattern has been found in populations of the rodent, *Ctenomys talarum* (Zenuto et al., 1999). The degree of territoriality could also affect testis size, with territorial males having higher access to mates and thus greater copulation rates during a breeding season (Olsson and Madsen, 1998). Finally, males belonging to species with greater clutch sizes may need to produce more sperm to be able to fertilise all the ova of a female (Emerson, 1997). Although each of these factors may play some role in most species, in general they each explain very little of the observed variation in relative testis size across species. More recently, a large number of studies have shown that perhaps the one single factor most strongly correlated with testis size is mating system.

Mating systems can be separated into three main categories (Emlen and Oring, 1977): monogamy (males and females form pair bonds); polygyny (one male mates with several females); and polyandry (one female mates with several males). In addition, it is possible for both males and females to have several partners during a single breeding season (polygynandry). Where the mating system is monogamous, males only need to produce sufficient sperm to fertilise their partner's eggs. This can be successfully achieved with the smallest relative testis size possible. Predictions concerning the testis size of polygynous males are more varied. On the one hand it has been argued that polygynous males should develop larger relative testes than monogamous males, as they need to mate more frequently and fertilise a larger number of eggs (the sperm depletion hypothesis): this pattern has been shown in several taxa (Cartar, 1985; Kappeler, 1997; Møller, 1988; Rising, 1996), but a number of other studies have not supported the sperm depletion hypothesis, showing that there is no difference in the relative testis size of males in monogamous and polygynous mating systems (Briskie, 1993; Harcourt et al., 1981; Harvey and Harcourt, 1984; Møller, 1991). Finally, it is argued that males

belonging to species with either polyandrous or polygynandrous mating systems will need to produce a greater quantity of sperm than either monogamous or polygynous males, as their ejaculates are in competition with those of rival males (the sperm competition hypothesis). Thus they are expected to have the largest relative testis size of all. This has been shown to be the case in a wide range of taxa (e.g. mammals [Dixson, 1987; Harcourt et al., 1981; Harcourt et al., 1995; Heske and Ostfeld, 1990; Kenagy and Trombulak, 1986; Short, 1979], birds [Briskie, 1993; Møller, 1991; Rising, 1996], amphibians [Jennions and Passmore, 1993; Kusano et al., 1991], fish [Stockley et al., 1997], and insects [Gage, 1994]).

Although the relationship between relative testis size and mating system has been widely studied across many taxa, reptiles (Class Reptilia) have been excluded from all such analyses. It has now been widely accepted that in all other groups analysed to date, relative testis size may be used as a predictor of mating system (Harcourt et al., 1995). Since there is currently no information available concerning the mating system of any species of New Zealand gecko (Family Gekkonidae), in this chapter I analyse variation in relative testis size within New Zealand geckos and use this information to make predictions about the mating systems of these species. I have also collected data from the literature on relative testis size in other species of lizards and snakes (Squamata), and related this to mating system where this has been reported. This information is then compared with that obtained for the New Zealand species to determine whether they differ from species elsewhere in the world.

A second, independent predictor of mating system is the degree of sexual size dimorphism (SSD), which reflects the summed effect of both natural and sexual selection acting upon each sex (Darwin, 1871; Selander, 1972). The degree of SSD observed in species has been related to several factors, such as body weight (SSD greater in larger species) (Clutton-Brock et al., 1977), activity period (SSD greater in diurnal species) (Vitt, 1986), and habitat (SSD greater in terrestrial species) (Clutton-Brock et al., 1977). However, it has also been shown that the mating system of a species can affect the amount of SSD. In general, species with non-monogamous mating systems tend to have a greater degree of SSD than monogamous species (Selander, 1972). The strongest sexual selection is believed to occur in polygynous species, where males are often much larger than females (Clutton-Brock et al., 1977; Heske and Ostfeld, 1990; Ralls, 1977;

Short, 1979; Stamps, 1983). In lizards it has been found that both body size and the dimensions of the head are often under strong sexual selection (e.g. Anderson and Vitt, 1990; Carothers, 1984; Hews, 1990; Vitt and Cooper, 1985). Therefore, I have measured intersexual variation in these features among New Zealand geckos, to make predictions about the mating system of each species. I then compare these predictions with those made from the testis size data.

Methods

Testis Size

a) New Zealand geckos

I collected measurements of testis length and width (for both the left and right testes) and snout-vent length (SVL) for 19 species from museum specimens held at the Museum of New Zealand Te Papa Tongarewa and Canterbury Museum (Appendix A). I also obtained data on testis mass where possible. Specimens had been fixed in formalin at some point in their history, although the duration of fixation and concentration of fixative were unknown. All had subsequently been stored in 70% ethanol or 40% isopropanol.

I calculated the volume for the left and right testis of each individual, using the formula for the volume of an ellipsoid (Mayhew, 1963): $\frac{4}{3} \pi ab^2$ (where $a = \frac{1}{2}$ the longest axis, $b = \frac{1}{2}$ the shortest axis). Volume was selected because I obtained little data on testis mass, and length alone is a less accurate predictor of size as variation in testis width is ignored. In addition, Mayhew (1966) showed that testis volume was directly correlated with breeding condition in the lizard *Uma notata*. I investigated whether any left-right asymmetry in testis size existed for the gecko species studied. This enabled me to determine whether one side should be used in the analyses, or whether a mean value should be calculated.

Very little information is available about size at maturity of New Zealand lizards. Therefore, I plotted testis volume against SVL for each species in order to determine whether there were any outliers at small body sizes, as would be expected for sexually immature individuals. No such effect was seen for any of the individuals collected. Consequently, all males with obvious testes and convoluted epididymides were assumed to be sexually mature for the purposes of these analyses.

Wherever possible, only males collected during the mating season were used (see Appendix A for mating season dates). However, this was not always possible both due to the paucity of samples for many species and because the exact mating season for some species is not well known. Furthermore, the accuracy of collection date is unknown, as it is possible that dates recorded by the museums are accession dates rather than collection dates for some specimens (Cree A, pers. comm.). Previous studies have varied in their conclusions about seasonal changes in testis size in New Zealand lizards (MacAvoy, 1976; McIvor, 1972; Robinson, 1985). Therefore, I also made measurements of testis size from a large collection of one species, *Hoplodactylus maculatus*, where individuals from Turakirae Head had been collected throughout the year by A.H. Whitaker (see Whitaker (1982) for a description of the study site and collection methods). From these samples, I determined variation in testis size according to season in this species, and made the assumption that other species would vary to a similar extent, given the similarity of environmental conditions and latitude of each. Robinson (1985) previously analysed this collection for the same purpose. However, this study failed to account for variation with respect to body size.

b) Lizards and snakes from other regions

I searched the literature for data on testis size and body size in as many species of lizards and snakes as possible. In total I collected information from 82 papers. However, only 33 of these were of use in my final analysis. Often there were inconsistencies in the usage of the words ‘testis’ and ‘testes’, so that in 31 of the papers I was unable to determine whether values presented were measures of one or both testes. This appears to be a common problem encountered in literature reviews of this nature (e.g. see Kenagy and Trombulak, 1986), and can only be rectified by more careful reporting of methodology in the future. In order for data to be included in the analysis I required unambiguous information on absolute testis size and body size for each species. These were generally both obtained from the same paper. However, on occasion body size information was gathered from other sources. If a range of body sizes was provided, I used the median value as an estimate of the average. Where there was seasonal variation in testis size, I used maximal values. Both fresh and preserved material were combined for the purposes of this analysis, as it has previously been found that there is very little

or no change in the size or shape of testes following fixation (Amey and Whittier, 2000; Hosken, 1998; Vitt, 1983).

In the literature, there are three main measures of testis size reported: length, volume and mass. Where both length and width were provided, I calculated the volume of one testis or mean volume using the equation for an ellipsoid (see above). I obtained testis size information using more than one measure for several species (particularly the New Zealand gecko species). Using weighted regression analysis, I found that there were significant positive relationships between both length and volume ($\log_{10} \text{Vol (mm}^3\text{)} = 1.99 \log_{10} \text{Length (mm)} - 0.07$, $F_{1,27} = 63.13$, $r^2 = 0.70$, $P < 0.001$) and mass and volume ($\log_{10} \text{Vol (mm}^3\text{)} = 0.69 \log_{10} \text{Mass (g)} + 2.56$, $F_{1,13} = 15.66$, $r^2 = 0.55$, $P = 0.002$). Consequently, I converted all length and mass measurements reported in the literature to volumes, using these equations. In addition to collecting data on testis size and body size, I also recorded the following information for each species, where possible: seasonality of breeding, sex ratio, clutch size and mating system. The entire data set is presented in Appendix B.

c) Data analysis

Testis volume was regressed against SVL for both groups. \log_{10} transformations were used to normalise the data. Weighted regressions were calculated using RGui, Version 1.7.0 (using the inverse of the relative influence of each data point as its weight) to minimise the influence of outliers on the slope and intercept of the line. Residual values for testis volume were then calculated from the weighted linear regression equation and compared between species. In the larger cross-species comparison, testis size residuals were correlated with each of the factors mentioned above, to see which, if any, could best explain the observed variation.

To ensure that demonstrated relationships in comparative studies are a result of convergent evolution rather than simply due to common ancestry, it is important to control for phylogenetic effects (Harvey and Pagel, 1991). Consequently, I controlled for phylogeny in all analyses for which a significant relationship was detected with the raw or transformed data. I used the ‘contrast method’, which examines changes between species since they last shared a common ancestor (Purvis and Rambaut, 1994). The taxonomy of New Zealand geckos is currently under review, as it is believed that one

species, *H. maculatus*, is a species complex (Hitchmough, 1991, abstract; Hitchmough, 1997). Consequently, populations of this species from different localities were treated as separate species in all analyses and referred to as *H. maculatus* “locality”. For the New Zealand geckos, I used a phylogeny constructed by Hitchmough (1997), generated from allozyme data and using Nei’s D method. Unfortunately one species (*H. maculatus* “Canterbury”) was absent from this phylogeny; consequently it was excluded from these analyses. For the larger cross-species comparison of lizards and snakes, a phylogeny was constructed using trees and information presented by Zug et al. (2001). Contrasts were calculated using Comparative Analysis by Independent Contrasts (CAIC) software programme, Version 2 (Purvis and Rambaut, 1994). In all cases it was assumed that branch lengths were equal. I then performed regression analysis on the contrasts to test the relationship in the absence of phylogenetic effects. Regressions were forced through the origin, as recommended by Purvis and Rambaut (1994). In some cases the evolutionary assumptions of these analyses were violated. However, I present results from both these phylogenetic contrasts and the raw data; where these are similar it is likely that the observed patterns are real and not a result of inaccurate procedures.

Sexual Size Dimorphism in New Zealand Geckos

Measurements of SVL, head length (tip of snout to anterior margin of ear opening) and maximum head width and depth were collected for 14 species (indicated in Appendix A) from male and female specimens held at the Museum of New Zealand Te Papa Tongarewa and Canterbury Museum. I decided not to use body weight as a variable in any of my analyses due to the many factors contributing to its variation, including female reproductive condition and the presence/absence of the tail which is autotomised in these species. I included in my analyses all individuals for which the sex could easily be determined: for males this meant that the testes had to be well developed with convoluted epididymides, and for females the ovaries contained developing follicles. It was assumed that the minimum size at maturity was the same for both sexes. Since sample sizes were small, no attempt was made to separate populations of the same species on the basis of collection locality, despite there being great geographical variation in body size for some species (Cree, 1994). However, males and females were relatively evenly distributed across localities (Appendix A), so any such effect should be negligible.

For each species, intersexual differences in body length were determined using t-tests. Head length, width and depth are not independent measures, as they are likely to be correlated with each other within an individual. Therefore I used multivariate analysis of covariance (MANCOVA) to determine differences in head dimensions between the sexes. I used SVL as a covariate to account for any variation that may exist purely as a result of differences in body size. Data were \log_{10} transformed where necessary to normalise the residuals and make variances homogeneous.

Results

Testis Size

a) New Zealand geckos

I determined the difference in volume of the left and right testes for individuals of each species using paired t-tests. No significant differences were found between the testes for any species. Comparison of testis size asymmetry across species showed that the right testis was larger than the left in 66.7% of species ($n = 18$) (paired t-test: $t_{17} = -2.14$, $P = 0.047$). However, the direction of this asymmetry was variable between species (Fig. 2.1). Consequently, mean testis volume was used in all subsequent analyses to avoid bias. In some individuals only one testis was present or in a suitable condition for measuring. Since there was no obvious bias in which testis was available, these values were still included in the analyses when calculating a mean value for the species.

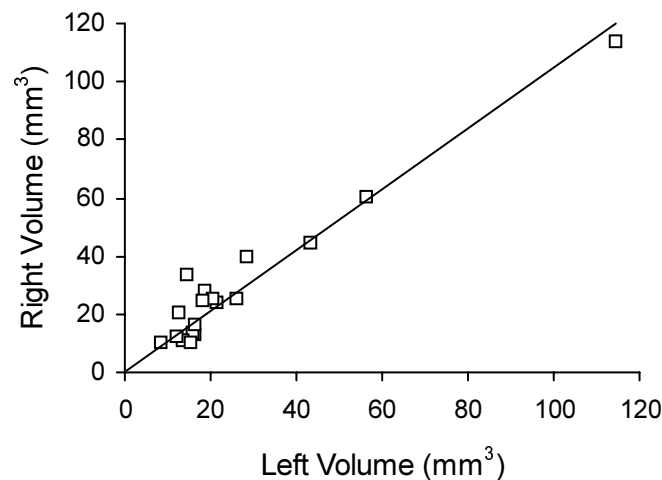


Fig. 2.1: Relationship between left and right testis volume in 18 gecko species. Line represents expectation if both testes of equal volume ($x = y$).

In *Hoplodactylus maculatus*, mean testis volume was significantly positively related to SVL ($F_{1,80} = 46.40$, $r^2 = 0.36$, $P < 0.001$). Consequently, all volumes were corrected for body size. Analysis of mean testis volume residuals revealed that although there was some variation in testis size between months, with minimum values in winter (August: mean volume = 7.29 mm^3) and maximum values towards the end of summer (March: mean volume = 11.67 mm^3) (Fig. 2.2), this difference only approached significance ($F_{9,72} = 1.99$, $P = 0.052$), and overall the size of the testes remained fairly constant between October and May. The relationship between mean testis volume residual and month was also analysed for all other species studied. Although there was insufficient data to statistically test any variation, there was no consistent relationship between mating season and relative testis size: during the mating season four species had larger relative testis size, two species had smaller relative testis size and four species showed no change in testis size when compared with values during the rest of the year. Consequently, for the purposes of the following analyses all individuals were included, irrespective of their collection date.

There was a significant positive relationship between mean testis volume and SVL for the 19 gecko species analysed (both variables \log_{10} transformed: $F_{1,17} = 7.29$, $r^2 = 0.30$, $P = 0.02$) (Fig. 2.3). This relationship became more significant when phylogenetic effects were controlled ($F_{1,14} = 23.51$, $r^2 = 0.63$, $P < 0.001$). There was great variation in relative testis volume (corrected for body size) between species, however, with *H. granulatus* having the largest relative volume ($+0.28$; $\text{SVL} = 75.33$

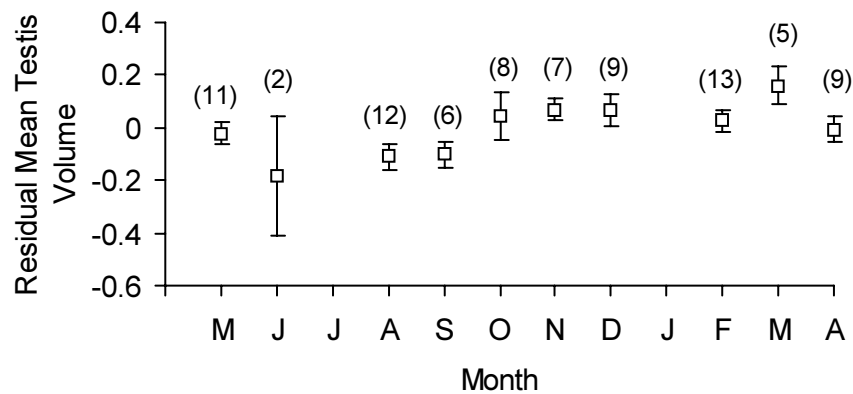


Fig. 2.2: Variation in residual testis volume (calculated from \log_{10} transformed data) between months for *Hoplodactylus maculatus* from Turakirae Head. Values represent mean \pm SE; sample sizes in parentheses.

mm, testis volume = 54.64 mm³) and *H. maculatus* “Three Kings” having the smallest (-0.38), despite its larger body size (SVL = 81.54 mm, testis volume = 14.63 mm³).

b) Lizards and snakes from other regions

In total I obtained information for 63 species of lizards and snakes, across 28 genera and 12 families (Appendix B). There was a significant positive relationship between mean testis volume and SVL across the 63 species analysed ($F_{1,61} = 33.20$, $r^2 = 0.35$, $P < 0.001$) (Fig. 2.4). Once again, this relationship became more significant after controlling for phylogeny ($F_{1,26} = 74.65$, $r^2 = 0.73$, $P < 0.001$). There was also considerable variation in relative testis size (corrected for body length) between species, however.

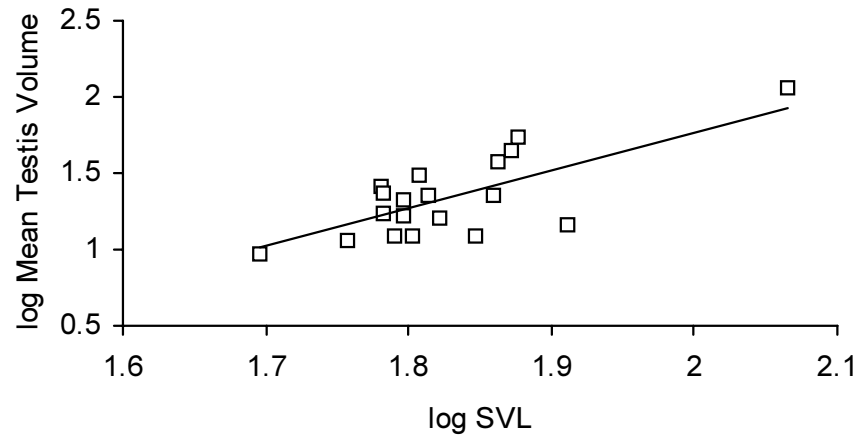


Fig. 2.3: Relationship between mean testis volume and SVL in 19 gecko species from New Zealand. Linear regression of \log_{10} transformed data: $\log_{10} \text{Vol} = 2.49 \log_{10} \text{SVL} - 3.22$, $r^2 = 0.30$.

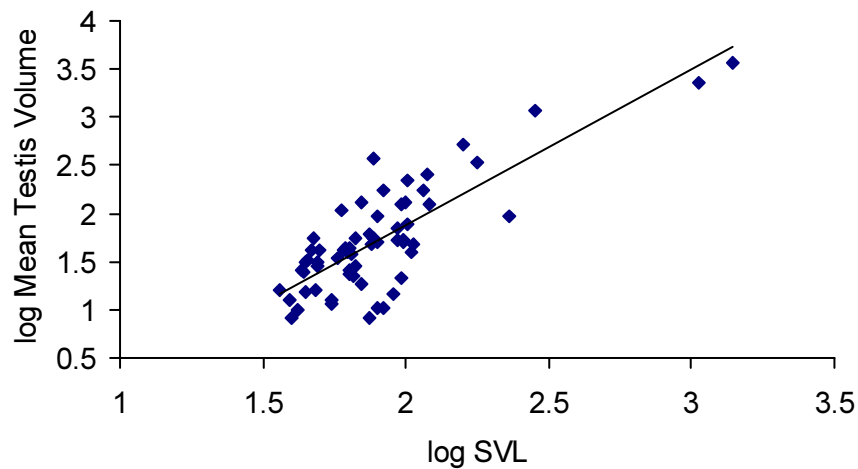


Fig. 2.4: Relationship between mean testis volume and SVL in 63 species of lizards and snakes. Linear regression of \log_{10} transformed data: $\log_{10} \text{Vol} = 1.62 \log_{10} \text{SVL} - 1.37$, $r^2 = 0.35$.

The minimum value was -0.74 for the lizard *Pletholax gracilis* (Family Pygopodidae), and the maximum was +0.88 for the snake *Austrelaps superbis* (Family Elapidae). These species are very similar in body size (75 mm and 76.6 mm SVL respectively), yet have more than a 40-fold difference in mean testis volume (8.38 mm³ and 369.30 mm³ respectively). This variation was not due to the condition of the testes, as there was no significant difference between fresh and preserved testes in terms of the number of positive and negative residuals ($\chi^2_1 = 1.79$, $P = 0.18$).

To explain some of this variation, I analysed whether any of the additional factors measured were correlated with testis volume residual. There was no significant difference in residual testis size between species that reproduced seasonally and those that reproduced all the year around ($t_{58} = -1.27$, $P = 0.21$), or species with male-biased, female-biased or even sex ratios ($F_{2,12} = 0.94$, $P = 0.42$). There was also no relationship between testis volume residual and clutch size ($F_{1,51} = 0.02$, $P = 0.90$).

Two species (*Anolis carolinensis* and *A. sagrei*) are reported in the literature to have polygynous mating systems (Jenssen and Nunez, 1998; Jenssen et al., 2000; Schoener and Schoener, 1980). *A. carolinensis* lies close to the regression line, with a residual value of -0.002. In contrast, *A. sagrei* lies above the line (testis volume residual = +0.29). *A. lineatopus* is reported to have a polyandrous/polygynandrous mating system (Olsson and Madsen, 1998, and references therein). This species also lies very close to the line (testis volume residual = +0.06).

c) Comparison of New Zealand geckos with other lizards and snakes

Compared with other species of lizards and snakes, 17 of the New Zealand gecko species had smaller mean testis volume than expected for their body size (range of residual values = -0.02 to -0.56) and two species (*H. duvaucelii* and *H. granulatus*) had testes slightly larger than expected (residual values = +0.09 and +0.07 respectively) (Fig. 2.5). The five gecko species from overseas had mean testis residual values ranging from -0.13 to +0.47. Six of the New Zealand gecko species fell within this range.

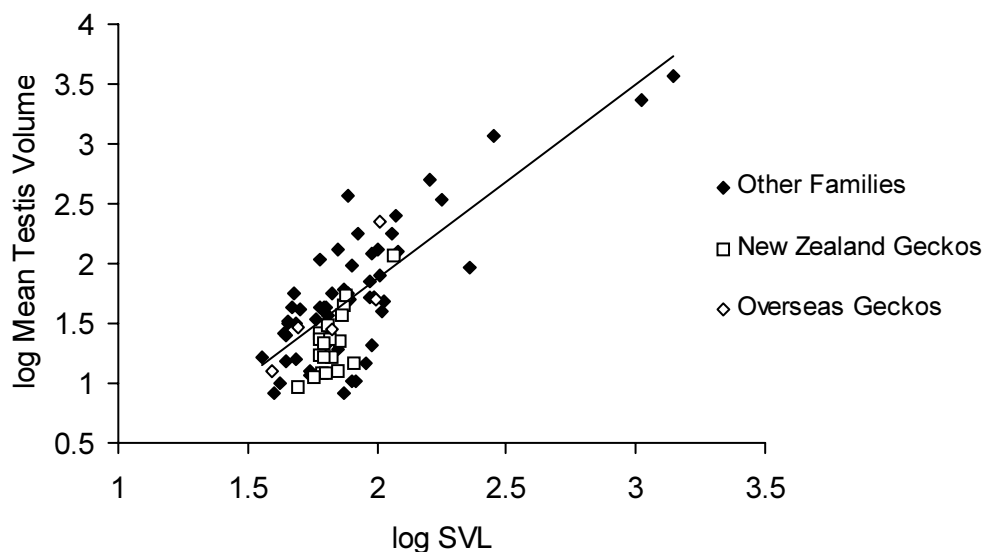


Fig. 2.5: Relative testis size of New Zealand geckos compared with lizard and snake species from other parts of the world. Regression line fitted to overseas species data.

Sexual Size Dimorphism in New Zealand Geckos

Sexual size dimorphism was analysed in 14 gecko species. There was a significant difference in SVL between the sexes for two species (*H. maculatus* “S. Alps” and *H. pacificus*) (Table 2.1). In both of these, males were significantly larger than females.

Multivariate analysis of covariance showed that in 11 species head dimensions were correlated with SVL (Table 2.2). In six species head dimensions differed significantly between the sexes when controlled for body size. In all of these, males were larger than females. There was no significant interaction between sex and SVL for any species.

Table 2.1: Sex differences in SVL (mean \pm SE) for 14 species of New Zealand gecko. Asterisk (*) indicates significant difference in SVL between males and females (t-test, $p < 0.05$). Ratio of male:female SVL (M:F ratio) also presented.

Species	Sex	Size range (mm)	n	SVL (mm)	M:F ratio
<i>Hoplodactylus duvaucelii</i>	M	106.3 – 125.1	11	116.38 \pm 1.742	1.01
	F	93.0 – 122.5	8	115.63 \pm 3.350	
<i>H. granulatus</i>	M	69.1 – 80.1	8	75.83 \pm 1.276	1.00
	F	70.4 – 81.5	12	75.75 \pm 1.009	
<i>H. maculatus</i>	M	59.7 – 68.6	8	63.45 \pm 1.281	1.08
	F	46.5 – 66.7	12	58.57 \pm 2.015	
“Canterbury” [§]	M	52.3 – 70.9	8	60.53 \pm 2.373	1.00
	F	51.0 – 70.9	11	60.68 \pm 2.113	
“E. Otago” [§]	M	53.0 – 72.9	7	66.76 \pm 2.492	1.07
	F	55.9 – 72.2	5	62.14 \pm 3.133	
“Marlborough” [§]	M	43.9 – 54.9	14	49.36 \pm 0.859	1.00
	F	43.9 – 55.3	13	49.35 \pm 1.005	
“Poor Knights” [§]	M	61.3 – 80.6	8	74.45 \pm 2.202	1.06
	F	66.4 – 74.9	9	70.40 \pm 1.082	
“Southern Alps” [§]	M	57.0 – 65.3	6	61.72 \pm 1.233 *	1.09
	F	48.6 – 64.6	14	56.48 \pm 1.153	
“Three Kings” [§]	M	75.5 – 86.2	5	81.54 \pm 2.483	1.03
	F	71.7 – 86.3	6	78.92 \pm 2.228	
<i>H. pacificus</i>	M	65.3 – 80.0	15	72.47 \pm 1.031 *	1.04
	F	67.0 – 72.4	5	69.40 \pm 0.868	
<i>Nautilinus elegans</i>	M	58.1 – 70.3	5	64.30 \pm 2.176	0.99
<i>elegans</i>	F	63.4 – 67.3	5	65.10 \pm 0.803	
<i>N. e. punctatus</i>	M	63.0 – 78.9	8	72.64 \pm 2.095	1.01
	F	59.7 – 80.4	9	71.67 \pm 2.199	
<i>N. gemmeus</i>	M	63.5 – 65.7	4	64.80 \pm 0.492	1.04
	F	66.5 – 80.4	5	71.24 \pm 2.474	
<i>N. manukanus</i>	M	59.2 – 62.0	5	60.48 \pm 0.570	0.97
	F	58.5 – 67.5	5	62.50 \pm 1.674	

[§] Members of the *Hoplodactylus maculatus* species complex

Table 2.2: Sex differences in head length, width and depth (mean (mm) \pm SE) for 14 species of gecko. Sample sizes in parentheses. Male:female size ratio (M:F) presented for each species. Asterisk (*) represents a significant difference between the sexes (MANCOVA with SVL as a covariate, $P < 0.05$).

Species	Measure	Sex			MANCOVA					
		Male	Female	M:F	Source	df	“Pillai”	~ F	df	P
<i>Hoplodactylus duvaucelii</i>	Length	30.63 \pm 0.449 (10)	30.23 \pm 0.911 (8)	1.01	Sex	1, 14	0.131	0.601	3, 12	0.626
	Width	25.69 \pm 0.631	25.18 \pm 1.103	1.02	SVL	1, 14	0.711	9.825	3, 12	0.001*
	Depth	16.56 \pm 0.542	16.59 \pm 0.871	1.00	Sex x SVL	1, 14	0.158	0.751	3, 12	0.543
<i>H. granulatus</i>	Length	18.78 \pm 0.322 (8)	18.40 \pm 0.202 (12)	1.02	Sex	1, 16	0.204	1.194	3, 14	0.348
	Width	15.00 \pm 0.311	14.70 \pm 0.224	1.02	SVL	1, 16	0.354	2.554	3, 14	0.097
	Depth	10.06 \pm 0.333	9.52 \pm 0.244	1.06	Sex x SVL	1, 16	0.228	1.382	3, 14	0.289
<i>H. maculatus</i>	Length	15.93 \pm 0.393 (8)	14.86 \pm 0.317 (12)	1.07	Sex	1, 16	0.646	8.511	3, 14	0.002*
	Width	12.74 \pm 0.428	12.44 \pm 0.310	1.02	SVL	1, 16	0.765	15.202	3, 14	<0.001*
	Depth	8.91 \pm 0.368	8.48 \pm 0.218	1.05	Sex x SVL	1, 16	0.358	2.604	3, 14	0.093
“Canterbury” §	Length	15.11 \pm 0.651 (8)	14.57 \pm 0.451 (11)	1.04	Sex	1, 15	0.438	3.379	3, 13	0.051
	Width	13.01 \pm 0.658	12.21 \pm 0.354	1.07	SVL	1, 15	0.945	74.581	3, 13	<0.001*
	Depth	9.74 \pm 0.612	9.20 \pm 0.277	1.06	Sex x SVL	1, 15	0.327	2.110	3, 13	0.148
“E. Otago” §	Length	16.49 \pm 0.511 (7)	14.98 \pm 0.695 (5)	1.10	Sex	1, 8	0.750	5.989	3, 6	0.031*
	Width	14.04 \pm 0.514	12.88 \pm 0.644	1.09	SVL	1, 8	0.867	13.027	3, 6	0.005*
	Depth	8.99 \pm 0.507	8.76 \pm 0.496	1.03	Sex x SVL	1, 8	0.100	0.222	3, 6	0.878
“Marlborough” §	Length	12.19 \pm 0.209 (14)	12.06 \pm 0.192 (13)	1.01	Sex	1, 23	0.347	3.723	3, 21	0.027*
	Width	10.70 \pm 0.144	10.18 \pm 0.166	1.05	SVL	1, 23	0.493	6.796	3, 21	0.002*
	Depth	7.64 \pm 0.193	7.37 \pm 0.214	1.04	Sex x SVL	1, 23	0.007	0.052	3, 21	0.984
“Poor Knights” §	Length	18.96 \pm 0.414 (8)	17.61 \pm 0.283 (9)	1.08	Sex	1, 13	0.674	7.562	3, 11	0.005*
	Width	15.91 \pm 0.470	14.98 \pm 0.298	1.06	SVL	1, 13	0.799	14.527	3, 11	<0.001*
	Depth	10.71 \pm 0.413	9.57 \pm 0.171	1.12	Sex x SVL	1, 13	0.306	1.618	3, 11	0.242

Table 2.2 Contd.

Species	Measure	Male	Female	M:F	Source	df	“Pillai”	~ F	df	P
“Southern Alps” §	Length	14.83 ± 0.314 (6)	13.78 ± 0.270 (14)	1.08	Sex	1, 16	0.693	10.513	3, 14	<0.001*
	Width	12.82 ± 0.362	11.54 ± 0.322	1.11	SVL	1, 16	0.867	30.462	3, 14	<0.001*
	Depth	8.47 ± 0.267	7.58 ± 0.271	1.12	Sex x SVL	1, 16	0.021	0.102	3, 14	0.958
“Three Kings” § †	Length	21.00 ± 1.114 (5)	20.03 ± 0.631 (6)	1.05	Sex	1, 7	0.539	1.951	3, 5	0.240
	Width	17.80 ± 0.855	16.67 ± 0.593	1.07	SVL	1, 7	0.888	13.146	3, 5	0.008*
	Depth	11.46 ± 0.538	10.77 ± 0.323	1.06	Sex x SVL	1, 7	0.334	0.836	3, 5	0.529
<i>H. pacificus</i>	Length	18.04 ± 0.248 (15)	16.90 ± 0.141 (5)	1.07	Sex	1, 16	0.818	20.963	3, 14	<0.001*
	Width	14.79 ± 0.178	13.46 ± 0.112	1.10	SVL	1, 16	0.857	27.881	3, 14	<0.001*
	Depth	9.83 ± 0.18	8.96 ± 0.341	1.10	Sex x SVL	1, 16	0.289	1.897	3, 14	0.177
<i>Nautilinus elegans</i>	Length	15.42 ± 0.375 (5)	15.42 ± 0.146 (5)	1.00	Sex	1, 6	0.590	1.918	3, 4	0.268
	Width	12.76 ± 0.652	13.22 ± 0.766	0.97	SVL	1, 6	0.904	12.568	3, 4	0.017*
	Depth	9.58 ± 0.304	9.14 ± 0.583	1.05	Sex x SVL	1, 6	0.472	1.194	3, 4	0.418
<i>N. e. punctatus</i>	Length	17.53 ± 0.415 (8)	17.50 ± 0.413 (9)	1.00	Sex	1, 13	0.027	0.100	3, 11	0.958
	Width	14.56 ± 0.502	14.32 ± 0.377	1.01	SVL	1, 13	0.808	15.471	3, 11	<0.001*
	Depth	10.03 ± 0.305	9.97 ± 0.266	1.01	Sex x SVL	1, 13	0.029	0.111	3, 11	0.952
<i>N. gemmeus</i>	Length	15.80 ± 0.449 (4)	16.04 ± 0.425 (5)	0.99	Sex	1, 5	0.775	3.437	3, 3	0.169
	Width	12.73 ± 0.409	13.38 ± 0.553	0.95	SVL	1, 5	0.889	7.992	3, 3	0.061
	Depth	8.78 ± 0.338	9.40 ± 0.387	0.93	Sex x SVL	1, 5	0.577	1.365	3, 3	0.402
<i>N. manukanus</i>	Length	14.57 ± 0.333 (3)	14.90 ± 0.277 (5)	0.98	Sex	1, 4	0.530	0.751	3, 2	0.615
	Width	11.70 ± 0.493	11.66 ± 0.252	1.00	SVL	1, 4	0.682	1.427	3, 2	0.437
	Depth	8.37 ± 0.273	8.88 ± 0.297	0.94	Sex x SVL	1, 4	0.604	1.017	3, 2	0.531

§ Members of the *Hoplodactylus maculatus* species complex† MANCOVA performed on log₁₀ transformed data (SVL and head length, width and depth)

Discussion

In birds and mammals, there is often marked asymmetry in testis size, with the left testis generally being larger than the right (Friedmann, 1927; Møller, 1994; Rising, 1996; but see Merila and Sheldon, 1999; Riddle, 1924). In contrast, no such consistent asymmetry has been observed in reptiles, so that whether researchers report values for the left, right or average of the two appears to be an arbitrary decision, and in general the assumption that both testes are equal in size appears justified (Olsson and Madsen, 1998). An explanation for this difference could lie in the fact that lizards and snakes have two intromittent organs (hemipenes), each linked to its own testis. Consequently, when they mate, one testis provides a complete ejaculate to the female. In contrast, in other vertebrates there is only one penis which is supplied by both testes. My finding, that several species of New Zealand geckos tend to have larger right testes than left, was unexpected. It is possible that in these species there may be a directional bias towards inserting the right hemipenis during copulation, as found in gartersnakes (*Thamnophis sirtalis*) (Shine et al., 2000b). Overall most species had relatively symmetrical testes, suggesting that no such bias is likely, as is the case in several species of anoline lizards (Crews, 1977).

In all temperate lizard species studied to date there is a marked seasonal change in the size of testes, with a reduction in size during the cooler winter months or period of hibernation and an increase during the spring (e.g. Crews, 1975; James, 1991; Jones et al., 1997; Sanyal and Prasad, 1967). The lack of any such seasonality in testis size in New Zealand geckos matches previous findings (McIvor, 1972; Robinson, 1985). Furthermore, it has been shown that spermatocytogenesis is continuous and thus sexual activity in males never fully declines (McIvor, 1992; Robinson, 1985; Saint Girons and Newman, 1987). The reason for this is unclear, as in all species there appears to be a defined mating season: during the spring for diurnal geckos (predominantly members of the genus *Naultinus*) and the autumn for the nocturnal species (*Hoplodactylus* spp.) (Gill and Whitaker, 1996; Robinson, 1985; Rowlands, 1981). Ovulation occurs during the spring in all species (MacAvoy, 1976; Whitaker, 1976). Therefore, there appears to be little obvious advantage to males in investing in the maintenance of enlarged testes continuously. A similar pattern has also been reported for *Pogona barbata* (Amey and Whittier, 2000), an agamid lizard found in Queensland, Australia. Males of this species

have an extended reproductive cycle with testicular regression restricted to one month. Amey and Whittier (2000) suggested that this may simply reflect a low cost of spermatogenesis. However, Olsson et al. (1997) demonstrated that there are substantial costs associated with testis enlargement and sperm production in male adders (*Vipera berus*). A second possible explanation for the retention of enlarged testes and extended period of sexual activity could be that it enables males to produce a greater volume of sperm more rapidly and thus gain an advantage in sperm competition (Amey and Whittier, 2000). It is evident that this finding is worthy of further investigation in the future.

There was a large variation in relative testis size between species of New Zealand gecko. The magnitude of this difference is quite surprising, as all species have very similar ranges and life-histories: New Zealand spans a relatively narrow latitudinal range (temperate to sub-tropical), so that although there is some North-South variation in breeding times, all species are seasonal breeders; in addition, members of the family Gekkonidae are constrained to a clutch size of two. Consequently, these factors cannot explain the observed variation. Variation of this magnitude is similar to or greater than that found in some mammalian groups, in which all mating systems are represented (Harcourt et al., 1995; Heske and Ostfeld, 1990; Hosken, 1998).

Even greater variation in relative testis size was observed for other lizard and snake species. Although there was great variation between species in latitude, and thus seasonality of breeding, clutch size and sex ratio, none of these factors significantly explained any of the observed variation in testis size. Consequently, the only remaining likely explanation is variation in mating system. Although it has been shown that polygynandry is widespread amongst reptiles (see review by Olsson and Madsen, 1998), the exact mating system is known for relatively few species. This is reflected by the fact that I was only able to define the mating system for three of the species used in this study, *Anolis sagrei*, *A. carolinensis* and *A. lineatopus*. The first two of these are both reported as being polygynous (Jenssen and Nunez, 1998; Jenssen et al., 2000; Schoener and Schoener, 1980), and have residual values falling in the middle of the observed range. In contrast, *A. lineatopus* is reported as having a polyandrous or polygynandrous mating system (Olsson and Madsen, 1998, and references therein). Consequently, this species would be expected to have greater residual testis volume than the previous two. However, it actually falls between the two, lying very close to the value expected for its body size.

The degree of sexual size dimorphism (SSD) is another morphological variable that is often correlated with mating system in animals: a larger disparity between the sexes can be indicative of greater sexual selection on the larger sex (Darwin, 1871). Thus, in polygynous species males are often larger than females (Heske and Ostfeld, 1990; Selander, 1972; Stamps, 1983), whereas in polyandrous species the reverse situation might be expected. Two aspects of lizard morphology believed to have evolved under sexual selection are body size and the dimensions of the head (Anderson and Vitt, 1990; Carothers, 1984; Hews, 1990; Vitt and Cooper, 1985).

In two species of New Zealand gecko, *H. maculatus* “S. Alps” and *H. pacificus*, males had significantly larger SVL than females, suggesting that these species may be polygynous. In all remaining species, there was no significant difference between the sexes in SVL. It has been argued, however, that SSD in body size may be a result of several factors other than sexual selection, such as intersexual differences in mortality rates and energetic demands (Carothers, 1984). In contrast, there is no reason to believe that these factors should affect head size in lizards: larger heads are believed to be important in male-male competition (Anderson and Vitt, 1990; Carothers, 1984; Gvoždík and Van Damme, 2003; Hews, 1990) and increase male mating success (Gvoždík and Van Damme, 2003). Thus, SSD in dimensions of the head may be a better predictor of the degree of sexual selection than overall body size. As expected, in the majority of species head dimension and SVL were highly correlated. Therefore, SVL needs to be taken into account when comparing the head dimensions of the two sexes. There was a significant difference between male and female head dimensions in six species, after controlling for SVL. In all six species, males had larger heads than females. However, there was no significant interaction between head size and SVL for any species, meaning that the gradient of the regression line was identical for both sexes, and thus head size increased with SVL at the same rate for each. This lack of difference is of significance, as it has been argued that this interaction effect is most important when determining whether sexual selection is occurring (Carothers, 1984; Cooper and Vitt, 1989). In addition, it can be seen from the ratios of male and female measurements for both SVL and each of the head dimensions that there is generally less than a 10% difference between the two sexes. Carothers (1984) interpreted a ratio of 1.1:1 as being significant SSD in herbivorous lizard species. Therefore, it can be concluded that there is little, if

any, SSD in head size in the New Zealand geckos, suggesting that in most species the intensity of sexual selection is approximately the same for both sexes. This indicates that most species are likely to have either monogamous or polygynandrous mating systems.

The strong relationship between relative testis size and mating system demonstrated across many other taxa enables us to use one to predict the other (Harcourt et al., 1995). Thus knowledge of the reproductive morphology of a species allows us to make predictions about its mating system. The conclusions from both the relative testis size data and the SSD results for head size suggest that most species of New Zealand gecko are either monogamous or polygynandrous. From information available for other reptiles, it seems unlikely that many, if any, of these species are monogamous, as this is very rare amongst reptiles (Bull, 2000). Indeed, in one species for which it has been reported, *Tiliqua rugosa* (Bull, 1988), it has recently been shown that extra-pair fertilisations can occur in up to 19% of females (Bull, 2000; Bull et al., 1998). Similarly, although it was once thought that the majority of reptiles were polygynous (Ruby, 1981; Stamps, 1983), it is now being found that females also have multiple partners in some species traditionally believed to be polygynous, such as *Uta stansburiana* (Zamudio and Sinervo, 2000). This highlights the fact that there is often some disparity between the observed social mating system of a species and its genetic mating system (Bull, 2000), and that in reality mating systems cannot be placed in discrete categories but are, instead, part of a continuum. Therefore, it could be the case that in the two species that have been identified as polygynous through observation of their social mating system in the cross-species comparison of testis size, females also have multiple partners, and the mating system is in fact polygynandrous. If this is the case, it is likely that the New Zealand geckos also have polygynandrous mating systems, as indicated through the SSD data. This seems a far more plausible conclusion, as it is currently believed that there is a high potential for sperm competition in reptiles, and the majority of reptiles probably have polygynandrous mating systems (Devine, 1984; Olsson and Madsen, 1998).

Conclusions

There is marked variation in testis size between species of reptiles. This variation is likely to reflect divergence in mating systems, in particular the degree of polyandry seen amongst females of a given species: species with greater relative testis size are likely to have a greater degree of polyandry than those with smaller relative testes. It seems likely that New Zealand geckos have polygynandrous mating systems, reflected through their lack of sexual dimorphism in head size. However, there is considerable variation in relative testis size even within this group of closely related species, suggesting interspecific variation in the intensity of sperm competition. It is evident that more in-depth studies on reptilian mating systems are required before any firm conclusions can be made from this cross-species comparison. However, the fact that there is so much variation in testis size within the Squamata suggests that this will be a very interesting study group for further investigation in the future.

Chapter 3: Male Reproductive Characteristics and Sperm Competition

Introduction

Males of closely related species often vary significantly in their reproductive morphology. One characteristic that has been of particular focus is the morphology of the intromittent organ of species with internal fertilisation, which often varies both in size and surface features (e.g. Arnold, 1986b; Dixon, 1987; Eberhard, 1990; Harcourt and Gardiner, 1994). Several theories have been proposed to explain this variation. Historically, the most favoured explanation was the ‘lock-and-key’ hypothesis, that males have evolved species-specific genitalia in order to prevent, or reduce the incidence of, interspecific matings. In some species there is evidence that divergent genital form does result in negative consequences to individuals involved in interspecific copulations. For example, Sota and Kubota (1998) found that in two species of carabid beetle there was an increase in both female mortality and the incidence of damage to the copulatory organs of males following interspecific matings. However, this theory currently has little support as a generalised explanation of genital variation, as not only are there often other, highly sophisticated mechanisms in place to prevent individuals coming into genitalic contact with members of different species, but large amounts of variation are often observed between closely related species that never come into contact with each other, whilst many species with overlapping ranges display little variation (Eberhard, 1990, 1993). A second suggestion is that these differences may arise through pleiotropy (Mayr, 1970): that is, selection acting upon other parts of a male’s phenotype result in changes in genital morphology through gene linkage; it is thought that genitalia may express these changes more readily than other parts of the body as they are less subject to natural selection and thus will have smaller associated costs (Arnold, 1973). There is evidence in some taxa that variation in genital morphology does appear to have evolved through pleiotropy. For example, in the water strider, *Gerris incognitus*, body size and the length of the first genital segment (a non-intromittent structure) were shown to be under sexual selection, resulting in a correlated increase in male genitalic morphology (Arnqvist et al., 1997). However, pleiotropy is also considered to be inadequate as a general explanation for the observed levels of variation in male intromittent organs, as it cannot

explain why similar variation is not observed in species with external fertilisation, nor why these effects occur so consistently in the genitalia (Eberhard, 1990).

More recently, theories implicating sexual selection have been proposed to explain interspecific variation in male genital morphology. Waage (1979) demonstrated that the penis of the damselfly, *Calopteryx maculata*, functioned not only in sperm transfer but also in sperm removal through the use of various morphological adaptations. This included features such as a flexible penile head with horns and backward pointing hairs at the base of the head. These traits enabled a male to scrape out sperm previously inseminated by rival males. Eberhard (1985) expanded upon this idea by suggesting that, as well as serving to transfer sperm, male intromittent organs may have the additional function of acting as internal courtship devices. Proposed mechanisms by which this could be achieved not only included the removal of sperm of rival males, but also the placement of a male's own sperm in a superior position within the female reproductive tract, holding the female more successfully and stimulating the female. Suggested morphological adaptations of the penis that may aid in this male-male competition are increased overall size and additional structures on the surface, such as spines, bristles and hairs. Since then, it has been shown that such mechanisms are found in several taxa. For example, spines have been implicated in the removal of sperm both directly (Gage, 1992; Haubruge et al., 1999) and indirectly, through stimulation of the female (Córdoba-Aguilar, 1999), and also in the ability of the male to accurately orient himself with respect to the female (Dixson, 1991). The shape of the penis has also been shown to affect sperm precedence (Arnqvist and Danielsson, 1999) and overall fertilisation success (Danielsson and Askenmo, 1999; House and Simmons, 2003). Thus there are several lines of evidence suggesting that postcopulatory courtship may exist in a wide range of taxa (also see Danielsson (1998) for a review of these processes in insects). Several evolutionary models have been implicated in this diversification of penis morphology. Patterns of genitalic evolution are largely consistent with Fisherian runaway evolution (Fisher, 1958), proceeding largely unchecked by natural selection until adaptations become debilitating in some way (Eberhard, 1985, 1993). In addition, females may directly discriminate between males based upon their genitalia (Eberhard, 1985), as found in the seedbug, *Lygaeus simulans* (Tadler, 1999). Finally, across a large number of taxa it has been shown that male genital morphology is strongly

correlated with mating system, with more elaborate surface features often being associated with species that have greater levels of sperm competition (e.g. in birds [Coker et al., 2002; McCracken, 2000], mammals [Brownell and Ralls, 1986; Dixson, 1987; Harcourt and Gardiner, 1994] and insects [Gage, 1992]).

Male squamates have two intromittent organs termed hemipenes. These are pocket-like structures that are stored retracted and in an inside-out position in the base of the tail; when everted, a groove on the surface (the sulcus spermaticus) transports sperm from the male's cloaca to the female (Cope, 1894, 1895; Harris, 1963; King, 1981). As in other taxa, a large amount of variation has been observed between closely related species in the morphology of these organs (e.g. Arnold, 1986a, b; Cope, 1896; Olsson and Madsen, 1998). Historically, studies investigating this variation have been conducted for taxonomic purposes (Arnold, 1973; Branch, 1981, 1986; Cope, 1894, 1895, 1896; Dowling and Savage, 1960), and although potential causes of interspecific variation have been discussed (Arnold, 1986b), sexual selection has largely been overlooked. In the single study that has addressed this issue, Olsson and Madsen (1998) found a positive correlation between the presence of spines on the hemipenis and copulation duration. However, they were unable to control for phylogeny in their analysis and did not investigate the relationship between hemipenis morphology and any other correlate of sexual selection or sperm competition intensity. Consequently, little is known of the relationship between hemipenial characteristics and sexual selection in reptiles. In this chapter I make a cross-species comparison of hemipenis size and surface features in the New Zealand geckos and relate hemipenis morphology to mating system, as indicated by relative testis size (see Chapter 2). I predicted that there would be diversification of hemipenis morphology across taxa according to relative testis size, with males belonging to species with larger relative testis size (and thus predicted to have more intense sperm competition) having longer intromittent organs with more complex surface features, such as folds, spines, bristles or hairs.

Sexual selection has also been implicated in explanations for interspecific variation in sperm morphology and ejaculate size. In several taxa, there is a positive correlation between testis size and the number or concentration of sperm produced (Breed, 1997; Møller, 1988, 1989) and testis size is, in turn, strongly related to mating system (see Chapter 2). In addition, in some species, the number of sperm produced or size of the

ejaculate has been directly correlated with the actual or perceived intensity of sperm competition or copulation frequency (Birkhead et al., 1993; Briskie, 1993; Evans et al., 2003; Gage and Barnard, 1996; Stockley et al., 1997; Svärd and Wiklund, 1989). It is widely believed that sperm competition should select for increased sperm production, so that males are able to inseminate more sperm per ejaculate (Parker, 1982, 1993); this enables males both to swamp or displace the sperm of rival males (e.g. Eady, 1995) and to reduce the likelihood that females will remate (Bissoondath and Wiklund, 1996, 1997; Eady, 1995; Letsinger and Gromko, 1985). In turn, it has been proposed that where this is the case, there should be concurrent selection for a reduction in sperm size, as males have a finite ability for sperm production (Dewsbury, 1982) and thus investment in number should trade-off against size (Parker, 1982; Parker and Begon, 1993). Although such a trade-off has been observed (Pitnick, 1996; Pitnick and Markow, 1994), contrary to prediction males exposed to intense sperm competition often instead invest their resources into increasing the size of their sperm rather than the number of sperm (e.g. Balshine et al., 2001; Briskie et al., 1997; Dixon and Birkhead, 1997; Gage, 1994; Gomendio and Roldan, 1991; LaMunyon and Ward, 2002; Morrow and Gage, 2000; Pitnick and Markow, 1994). In mammals it has been shown that longer sperm are capable of swimming faster (Gomendio and Roldan, 1991; although also see Gage et al. (2002) for a lack of this relationship in fish). In addition, Radwan (1996) demonstrated that sperm length was significantly correlated with fertilisation success in the bulb mite (*Rhizoglyphus robini*). Thus it is possible that male investment in sperm length may provide a selective advantage in reaching and fertilising the ova.

In previous comparative studies investigating sperm morphology in reptiles, interspecific variation has been analysed to elucidate taxonomy and phylogenetic relationships (e.g. Healy and Jamieson, 1992, 1994; Jamieson and Healy, 1992; Jamieson et al., 1996; Oliver et al., 1996). However, there has been no attempt to correlate interspecific variation in sperm morphology with ecological or life-history traits. Consequently, in the second section of this chapter I compare sperm length in the New Zealand geckos and relate this to mating system, using relative testis size as an index. I predicted that males belonging to species with larger relative testes would have longer sperm, enabling them to out-swim and thus out-compete rival males under sperm competition.

Methods

Hemipenis Morphology

a) Hemipenis size

Measurements were made both of everted hemipenes of live males and inverted organs of preserved males. Intuitively, the everted hemipenis should better reflect the dimensions of the organ during copulation and thus should be more strongly correlated with mating system. However, everted organ measurements were only available for relatively few taxa. In addition, the method I used to evert the organ, by applying pressure to the base of the tail, probably did not result in the hemipenis having the same dimensions as it would if everted voluntarily by the male. Consequently, to standardise the measurements obtained across males, I also determined the size of the inverted organ. Although this may not directly reflect the dimensions of the organ once everted, it could be obtained for a greater number of species and in a more systematic fashion.

Everted hemipenes of live males:

(i) Intraspecific variation:

Hemipenis measurements were obtained for 29 male *Hoplodactylus maculatus* “Canterbury”, collected from rock outcrops on the Port Hills, Christchurch, from April-June 2002, as part of a study on sperm morphology and motility (Chapters 6 & 7). Although this was not the optimal time for collecting sperm, as this species mates from February-May (MacAvoy, 1976; pers. obs. Chapter 5), sperm were still present in the vas deferens/epididymis during the sampled period. Both hemipenes were everted through manual pressure at the base of the tail. Measurements were made of the total length of the hemipenis, from the cloaca to the tip, and the width across the widest point (the tip) of the organ. Dimensions of the left and right hemipenis were compared and the degree of intraspecific variation in hemipenis size determined.

(ii) Interspecific variation:

Males belonging to the following species were obtained from Ti Point Reptile Park, Leigh, during October 2002: *H. duvaucelii*, *H. granulatus*, *H. pacificus*, *Naultinus elegans elegans*, *N. grayii*, and *N. stellatus*. Only 1-2 individuals were available for each species. Hemipenes were everted as outlined above. Although this is a relatively simple procedure, it is stressful for individuals not used to being handled. Consequently,

only the left hemipenis was measured in the majority of individuals, as this was often the first to be everted. Left hemipenis measurements for *H. maculatus* “Canterbury” were added to this data set to determine the amount of interspecific variation in hemipenis size. In addition, hemipenis size (corrected for snout-vent length (SVL) where necessary) was correlated with mean testis volume residuals for each species (Chapter 2) to see whether there was any relationship between the dimensions of the everted organ and mating system.

Inverted hemipenes of preserved males:

(i) Intraspecific variation:

Measurements were made of the inverted hemipenes of 12 preserved males belonging to *H. maculatus*, from a collection made by A.H. Whitaker at Turakirae Head (see Whitaker (1982) for a description of the study site and collection techniques). Hemipenes were taken from individuals collected at various times of the year (Month (sample size): April (2), May (3), August (2), October (1), November (3), and December (1)). Only the right hemipenis was measured for each individual, to minimise damage to the specimens. A superficial medial incision was made from the cloaca to the base of the hemipenial sacs, easily observed externally in male geckos by the presence of two enlarged regions at the base of the tail. The skin was then peeled back and any fat deposits lying above the hemipenis were gently removed. The hemipenis could then clearly be seen sitting within its thin sac. The total length of the organ and width across the apex were measured *in situ* (Fig. 3.1). From this, I determined the degree of intraspecific variation in the dimensions of the hemipenis and related this to time of year.

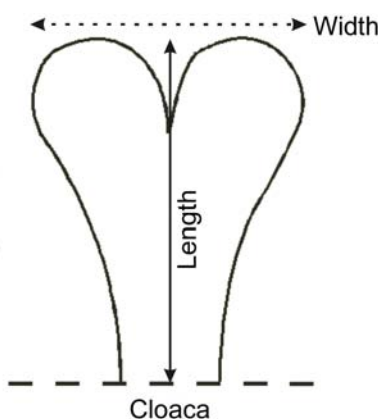


Fig. 3.1: Diagram of an inverted hemipenis showing the location of measurements.

(ii) Interspecific variation:

The inverted hemipenes of preserved males were measured *in situ*, using the methodology outlined above. Individuals were obtained from the Museum of New Zealand Te Papa Tongarewa and Canterbury Museum. The following 17 species were sampled: *H. duvaucelii*, *H. granulatus*, *H. maculatus*, *H. maculatus* “Canterbury”, *H. maculatus* “Eastern Otago”, *H. maculatus* “Marlborough”, *H. maculatus* “Poor Knights”, *H. maculatus* “Southern Alps”, *H. maculatus* “Three Kings”, *H. pacificus*, *N. e. elegans*, *N. e. punctatus*, *N. gemmeus*, *N. grayii*, *N. manukanus*, *N. rudis* and *N. stellatus*. For the majority of species only 1-2 males were available for dissection. Wherever possible individuals collected during the mating season were used (see Appendix A for mating season dates). However, this was not always feasible due to sample availability. Furthermore, it should be noted that the accuracy of collection date information is unknown, as some specimens in museum collections are marked with accession dates rather than collection dates (Cree A, pers. comm.). The dimensions of both hemipenes were measured in all individuals. However, there was no significant difference between the dimensions of the left and right hemipenis across species (paired t-tests - Length: $t_{13} = -0.76$, $P = 0.46$; Width: $t_{13} = -0.68$, $P = 0.51$). Consequently, since only the right hemipenis was measured to determine intraspecific variation in *H. maculatus* and the right hemipenis of specimens was used to compare surface morphology (see below), only measurements of the right hemipenis were used in all analyses. I used these measurements to investigate the degree of interspecific variation in hemipenis size and also to relate this to mating system, as indicated by relative testis volume.

b) Hemipenis surface morphology

Preparation of material:

(i) Fresh material:

Investigations of hemipenis morphology are best made on freshly prepared material, where it is possible to evert the hemipenis prior to preservation. This minimises the potential for artefacts and allows the natural shape of the organ to be studied. Fresh material was available for seven male *H. maculatus* “Canterbury” that died during the mating study (Chapter 5). In these individuals, hemipenes were everted shortly after death, prior to fixation: the inverted hemipenes were exposed, using the same technique

as used to measure dimensions of the inverted organ (see above); organs were then dissected out by severing the magnus retractor muscle at the posterior end and cutting through the anterior tissue as close to the cloaca as possible; following this, hemipenes were everted in distilled water, using round-tipped forceps and a blunt probe to push the lobes inside out.

Following eversion, the hemipenes were fixed in 2.5% glutaraldehyde and 1% paraformaldehyde in 0.1M sodium cacodylate for three days. Samples were then prepared for scanning electron microscopy (SEM) using the following procedure: washed overnight in 0.1M sodium cacodylate buffer; stained in 2% osmium tetroxide (OsO_4) in distilled water for two days; washed in distilled water and dehydrated through an ethanol series (30%, 50%, 70%, 80%, 90%, 95%, 100%) for a minimum of 2 h at each concentration; left overnight in absolute ethanol; placed in a four step increasing concentration gradient of ethanol/amyl acetate to pure amyl acetate, for a minimum of 2 h at each concentration; left overnight in pure amyl acetate; critical point dried with liquid CO_2 ; mounted on aluminium SEM stubs using conductive carbon paint; and sputter-coated with ca. 40 nm gold/palladium. Hemipenes were viewed using a LEICA S440 scanning electron microscope at accelerating voltages of 10-20 kV.

I initially found that hemipenes prepared in this way were covered with a layer of mucus (Duckett J, pers. comm.), giving them a smooth appearance and obscuring surface features (Fig. 3.2A). To remove this material it was necessary to soak the freshly everted organ in a protease buffered in 0.1M sodium cacodylate for 3 min. Hemipenes were then placed in an ultrasonic bath in 0.1M sodium cacodylate buffer for 2 min, to remove any residues of the material. Following this, the tissue was fixed and prepared for SEM as outlined above. This technique successfully removed the mucus to expose the underlying surface features (Fig. 3.2B).

(ii) Ethanol-stored specimens:

Right hemipenes were obtained from six male *H. maculatus* collected from Turakirae Head. These individuals had been placed directly into 70% ethanol upon collection, with no prior fixation. Consequently, the tissue was comparable to fresh material in terms of pliability and could be everted, fixed and prepared for SEM in the same way as outlined above. No mucus covered these organs; thus the protease treatment was not required.

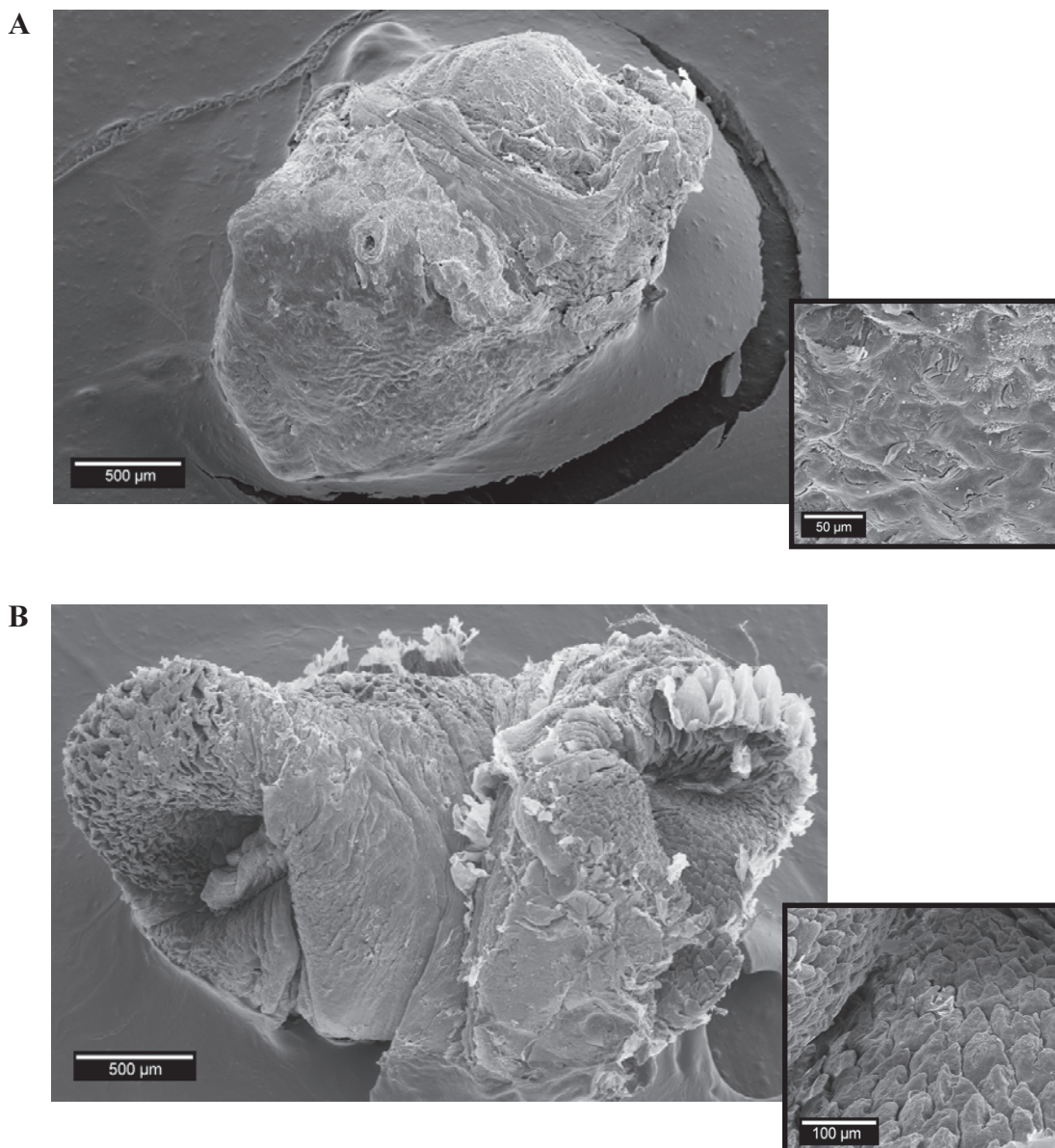


Fig. 3.2: SEM micrographs of everted hemipenes of *Hoplodactylus maculatus* “Canterbury”: A. Covered with mucus; B. Mucus removed following protease treatment. Insets show surface features at high magnification.

(iii) Formalin-fixed specimens:

Since fixation in formalin tends to harden tissue, it is difficult to evert fixed hemipenes without causing damage to their structure. As a result, it has been common for past researchers to simply dissect fixed organs *in situ* to investigate their surface features (e.g. Arnold, 1973, 1986a; Dowling and Savage, 1960). The disadvantage of this method is that it provides little insight into the overall structure of the everted organ. Consequently, I attempted to soften and evert the fixed organs.

Softened material using potassium hydroxide (KOH):

Pesantes (1994) described a method for softening preserved snake hemipenes, by soaking the material in a 2% solution of KOH for three days. I initially followed this methodology to soften the inverted left hemipenis of 16 museum specimens belonging to eight species of gecko obtained from the Museum of New Zealand Te Papa Tongarewa. The exact preparation history of the museum specimens was unknown, but all had been fixed in formalin (concentration and duration unknown) and stored in 70% ethanol. The specimens were between 19 and 35 years of age.

Hemipenes were first dissected in their entirety from the base of the tail, as outlined above. The samples were rehydrated from 70% ethanol to distilled water in a decreasing concentration gradient (50%, 30%), by soaking for a minimum of 1 h at each concentration. The hemipenes were then softened using KOH (Pesantes, 1994). I found that samples ($n = 6$) soaked in a 2% solution of KOH for three days either partially or totally disintegrated. Therefore, I modified this method and found that best results were obtained when the hemipenes were soaked in a 1% solution of KOH for 5-11 h. Following softening, they were rinsed in 0.05N HCl for 10 min and washed overnight in distilled water. Specimens were then everted in distilled water using round-tipped forceps and a blunt probe to push the lobes inside out. Once everted, samples were fixed and prepared for SEM using the methodology outlined above.

The condition of hemipenes prepared in this way was compared with those of the six specimens of *H. maculatus* that had been stored in ethanol and did not require any treatment prior to eversion (see above). The use of KOH to evert fixed hemipenes gave very variable results. From a total of 16 samples, four were everted in excellent condition (Fig. 3.3A), equal to that of the ethanol-stored specimens (Fig. 3.3B). However, for the remainder of the samples I found that the KOH treatment resulted in partial or complete loss of the surface layer of scales (Fig. 3.3C). This finding agrees with that of Glaw et al. (1999), who also found that the KOH treatment was sometimes too harsh for delicate organs. I therefore considered that a milder softening agent was required.

Saponin:

Previously it has been shown that detergents can be used to partially recover formalin-fixed material (Humason, 1962). Maupin and Pollard (1982) also demonstrated that the permeability of cell membranes to fixatives and stains could be improved by soaking

the tissue in saponin, a plant glycoside with detergent-like properties that is extracted from the bark of *Quillaja*. I tested whether saponin could soften formalin-fixed hemipenes and enable me to evert them more easily and with less damage than I experienced with the KOH treatment.

I dissected and rehydrated the right hemipenis of 22 specimens belonging to 10 species using the methods outlined above and then soaked them in a 1% solution of saponin in distilled water. The samples were checked at 15 min intervals to detect the best time for eversion. This was done by using a blunt probe to check the material for optimal softness and pliability. I found that the organs regained sufficient flexibility for eversion after 1-2 h in the saponin solution. The hemipenes were then everted, rinsed 3-5 times in distilled water over a 30 min period, and prepared for SEM as outlined above.

Saponin treatment yielded excellent results in 16 out of 22 samples (Fig. 3.3D), comparable to the most successful KOH-prepared specimens and the ethanol-stored

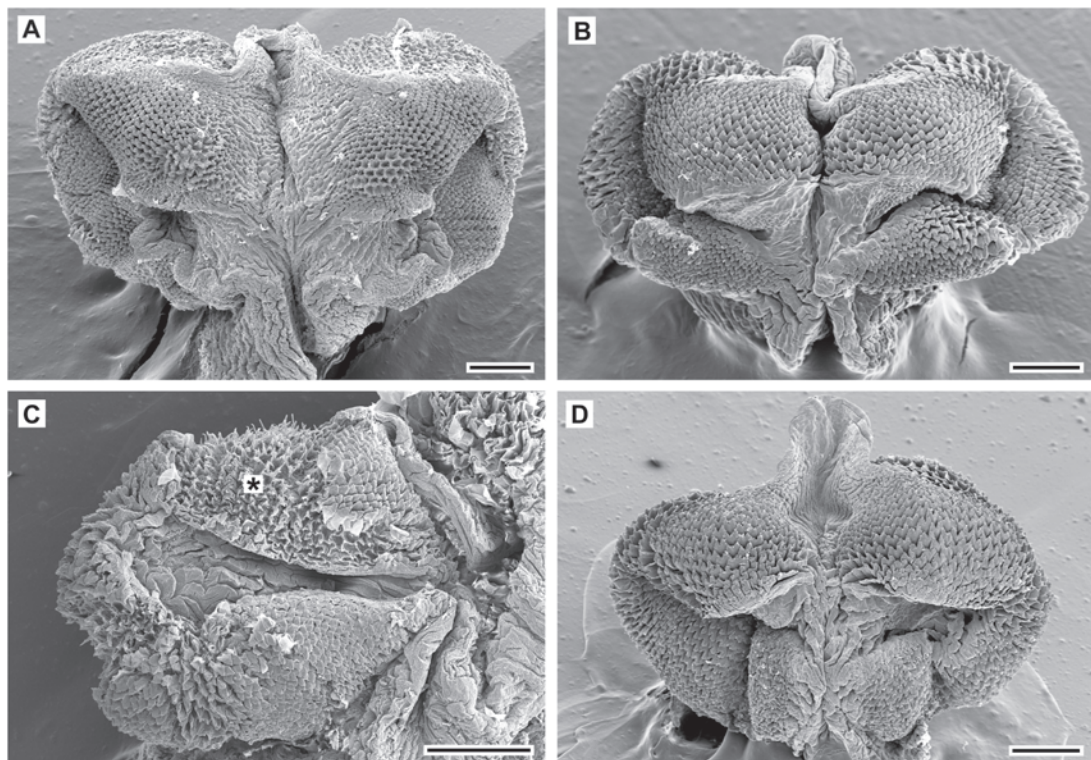


Fig. 3.3: SEM micrographs of the sulcal surface of hemipenes of *Hoplodactylus maculatus* prepared using different treatments: A. Example of a good specimen softened in potassium hydroxide; B. No treatment – ethanol stored sample; C. Single lobe of hemipenis damaged when softened in potassium hydroxide – note loss of calyces (*); D. Saponin-prepared sample in excellent condition. Scale bars = 500 μm .

samples. Although the minimum soaking time required to sufficiently soften the material varied between samples (presumably a function of their original collection/storage regime), there appeared to be no risk of over-softening the tissue and thus losing delicate surface features. Only in six samples did saponin fail to soften the organs sufficiently to evert them. In these instances, the tissue had been left in the solution for 2.5 h and appeared to have regained sufficient flexibility for eversion. However, upon attempting eversion I found that the material crumbled, as the tissue inside was still very hard and brittle. It may be that longer soaking times were required for these samples, or that some samples simply cannot be reclaimed using this method.

Intraspecific variation in hemipenis morphology:

Hemipenis morphology was compared between six *H. maculatus* individuals that had been collected at different times of the year to determine whether there was any seasonal variation in structure.

Interspecific variation in hemipenis morphology:

The morphology of hemipenes everted following the saponin treatment was compared between males belonging to 15 species (*H. duvaucelii*, *H. granulatus*, *H. maculatus*, *H. maculatus* “Canterbury”, *H. maculatus* “Marlborough”, *H. maculatus* “Poor Knights”, *H. maculatus* “Southern Alps”, *H. pacificus*, *N. e. elegans*, *N. e. punctatus*, *N. gemmeus*, *N. grayii*, *N. manukanus*, *N. rudis* and *N. stellatus*). In addition, the size and density of surface features were measured under SEM. Care must be taken when making measurements of structures in this way, as the angle at which the sample is tilted on the stage will affect the observed size of the structure. To make measurements as accurate as possible, the structures were oriented so they were in the same plane as the field of view. In addition, maximum possible measurement errors were calculated and compared with the observed degree of interspecific variation. The size of the surface features (corrected for SVL where necessary) was correlated with mean testis volume residuals, to determine whether they varied with mating system.

Sperm Morphology

a) Fixed sperm

I collected epididymal sections from preserved males belonging to 18 species (*H. chrysosireticus*, *H. duvaucelii*, *H. granulatus*, *H. maculatus*, *H. maculatus* “Canterbury”, *H. maculatus* “Eastern Otago”, *H. maculatus* “Marlborough”, *H. maculatus* “Poor Knights”, *H. maculatus* “Southern Alps”, *H. pacificus*, *N. e. elegans*, *N. e. punctatus*, *N. gemmeus*, *N. grayii*, *N. manukanus*, *N. rakiurae*, *N. rudis* and *N. stellatus*). Specimens were provided by the Museum of New Zealand Te Papa Tongarewa and Canterbury Museum. As in the hemipenis study, wherever possible samples were taken from individuals collected during the mating season. However, this was not always feasible due to the paucity of samples. Small sections of epididymis were placed in a drop of distilled water on a glass slide and teased apart under a Leica MZ6 stereomicroscope. These were then covered with a coverslip and viewed at a magnification of x400 with a Leica DMR microscope with phase-contrast illumination. The majority of sperm were measured directly using an ocular micrometer. However, in later samples more accurate measures of sperm length were made from digital images: recordings were made of the samples using a JVC TK-C1381 colour digital video camera attached to the microscope; still digital images were captured from these recordings using EthoVision Color-Pro, Version 3.0.8; and sperm length was measured using Image-Pro Plus, Version 4.5.0.19. Both methods were used for one species, *H. pacificus*, for which I found no significant difference in sperm length estimate between methods ($t_4 = -1.59$, $P = 0.19$). Consequently, estimates made using both techniques were combined for further analysis.

One problem encountered during this procedure was the breakage of sperm into fragments, presumably due to fixation making them brittle. Consequently, I avoided measuring obviously broken sperm and the maximum length of sperm measured was used in all analyses in an attempt to obtain measurements from the most intact sperm.

Interspecific variation in sperm length was analysed. To determine whether mating system influenced sperm length across species, sperm length was correlated with relative testis volume. Total sperm length was used in all analyses as it was difficult to distinguish the head, midpiece and tail precisely under the light microscope.

b) Fresh sperm

Fresh sperm samples were collected through manual palpation of the abdomen from males belonging to four species: *H. maculatus* “Canterbury”, *H. pacificus*, *N. grayii* and *N. stellatus*. *H. maculatus* “Canterbury” males were collected from the Port Hills, Christchurch, from April-June 2002 as part of an investigation into intraspecific variation in sperm morphology and motility (Chapters 6 & 7). Males of the other three species were obtained from Ti Point Reptile Park, Leigh, during October 2002. For the latter samples, sperm were stored in Dulbecco’s Modified Eagle’s Medium (Sigma Product No. D2902) prior to analysis, enabling me to transport them to the laboratory alive, and thus eliminating the risk of altering their morphology through preservation.

To measure sperm length, sperm samples were placed on a slide with a coverslip and viewed at x400 magnification with a Leica DMR microscope with phase-contrast illumination. Images of the sperm were recorded, still digital images captured and sperm length was measured, using the packages outlined above. Since sperm were still alive at the time of image capture, it was often difficult to obtain complete images of sperm where the whole sperm was in the same focal plane. Consequently, 10 sperm were measured for each individual and averages calculated.

In total, I obtained samples from eight male *H. maculatus* “Canterbury”, enabling me to determine the level of intraspecific variation in sperm length in this species. Only one male was available for each of the other species. Thus, these samples were used to analyse interspecific variation in sperm length. These results were compared with those obtained from the fixed sperm samples, to determine the accuracy of the fixed sperm estimates.

Data Analysis

Weighted regression analysis was used to reduce the influence of the outlier (*H. duvaucelii*) in the cross-species analysis of male reproductive morphology and body size (SVL). Where there was a significant relationship with SVL, residual values were calculated and used in all further analyses. In all statistical tests, residuals were tested for normality and homogeneity of variance; where these assumptions were violated, the data were transformed. Relative testis volume was calculated from the relationship between testis volume and SVL for New Zealand geckos (see Chapter 2).

I controlled for phylogeny in all analyses for which a significant relationship was detected using the ‘contrast method’ (Purvis and Rambaut, 1994), as outlined in Chapter 2. I used a phylogeny constructed by Hitchmough (1997), generated from allozyme data and using Nei’s D method, and assumed that all branch lengths were equal. Populations of the *H. maculatus* species complex arising from different geographic locations were treated as separate species and referred to as *H. maculatus* “locality”. Where necessary, data were transformed to meet evolutionary and statistical assumptions of this analysis.

Results

Hemipenis Morphology

a) Hemipenis size

Intraspecific variation:

(i) Everted hemipenes of live males (*Hoplodactylus maculatus* “Canterbury”):

I found no significant difference between the left and right hemipenes for either length (paired t-test: $t_{28} = 1.36$, $P = 0.19$) or width (paired t-test: $t_{28} = -0.84$, $P = 0.41$) of the everted organ. There was a significant positive relationship between left hemipenis length and SVL ($F_{1,27} = 4.80$, $r^2 = 0.15$, $P = 0.04$). This relationship was also strong (although not significant) for right hemipenis length ($F_{1,27} = 4.14$, $r^2 = 0.13$, $P = 0.052$). Width was not significantly related to SVL for either hemipenis (Left: $F_{1,27} = 1.59$, $r^2 = 0.06$, $P = 0.22$; Right: $F_{1,27} = 0.88$, $r^2 = 0.03$, $P = 0.36$). There was a large amount of variation between males in the length of their hemipenes when residual values (corrected for SVL) were compared (Left: +0.97 to -1.32; Right: +1.75 to -1.13), reflecting a variation of up to 167% in absolute size between males of similar body size. There was also great variation in hemipenis width between individuals (Left: 1.98-4.36 mm; Right: 1.94-4.42 mm).

(ii) Inverted hemipenes of preserved males (*H. maculatus*):

Right hemipenis size was not significantly related to SVL (Length: $F_{1,10} = 2.30$, $r^2 = 0.19$, $P = 0.16$; Width: $F_{1,10} = 0.38$, $r^2 = 0.04$, $P = 0.55$). Consequently, raw measurements were compared between individuals. Length measurements differed by up to 164% and there was up to a two-fold difference in widths between individuals. One-way ANOVA showed that there was no significant difference between months in

the dimensions of the hemipenes (Length: $F_{5,6} = 0.49$, $P = 0.78$; Width: $F_{5,6} = 0.23$, $P = 0.93$), suggesting that this variation is not a seasonal effect.

Interspecific variation:

(i) Everted hemipenes of live males:

There was no significant relationship between length or width of the everted left hemipenis and SVL in live males across seven species (Length: $F_{1,5} = 0.07$, $r^2 = 0.01$, $P = 0.81$; Width: $F_{1,5} = 0.03$, $r^2 = 0.006$, $P = 0.87$). There was a relatively large amount of interspecific variation in hemipenis size, with length varying by 138% (5.08-7.00 mm) and width by 168% (2.78-4.68 mm) between species. Hemipenis length was significantly positively correlated with mean testis volume residual ($r = 0.96$, $P = 0.001$) (Fig. 3.4). This relationship became more significant after controlling for phylogeny ($F_{1,4} = 125.67$, $r^2 = 0.97$, $P < 0.001$). There was no relationship between hemipenis width and mean testis volume residual ($r = 0.005$, $P = 0.99$).

(ii) Inverted hemipenes of preserved males:

There was a significant positive relationship between hemipenis size and SVL for both length and width across species (Length: $F_{1,15} = 18.97$, $r^2 = 0.56$, $P < 0.001$; Width: $F_{1,15} = 37.07$, $r^2 = 0.71$, $P < 0.001$). This relationship remained significant after controlling for phylogeny using log-log transformed data (Length: $F_{1,12} = 134.95$, $r^2 = 0.92$, $P < 0.001$; Width: $F_{1,12} = 55.77$, $r^2 = 0.82$, $P < 0.001$). Consequently, residuals were calculated and used in further analyses. Mean residual species values varied considerably for both measurements (Length: +0.96 (*Nautilinus grayii*) to -0.85 (*N. elegans punctatus*); Width: +0.42 (*H. granulatus*) to -0.91 (*H. maculatus*)).

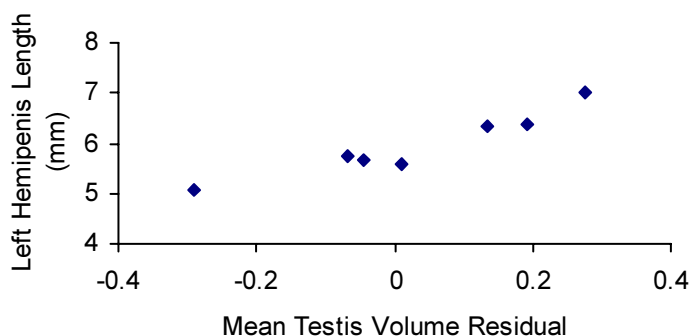


Fig. 3.4: Relationship between everted left hemipenis length and mean testis volume residual in seven species of New Zealand gecko.

There was no correlation between the dimensions of the left everted hemipenis (raw data) and right inverted hemipenis (controlled for SVL) across species (Length: $r = 0.02$, $P = 0.98$; Width: $r = 0.45$, $P = 0.31$).

To determine whether there was a relationship between hemipenis size and mating system across species, each measure was correlated with mean testis volume residual. Since not all individuals were collected during the mating season, I used analysis of covariance (ANCOVA) to determine whether dimensions varied between seasons and whether there was any relationship between dimensions and testis volume residual. There was no difference in residual hemipenis length or width between the mating and non-mating season (Length: $F_{1,11} = 0.02$, $P = 0.88$; Width: $F_{1,11} = 0.21$, $P = 0.66$) and no correlation between residual length or width of the inverted organ and mean testis volume (Length: $F_{1,11} = 0.03$, $P = 0.86$; Width: $F_{1,11} = 3.47$, $P = 0.09$). However, there was a significant interaction between season and testis volume residuals for both length and width of the right hemipenis (Length: $F_{1,11} = 7.54$, $P = 0.02$; Width: $F_{1,11} = 7.02$, $P = 0.02$). For both measures there was a positive relationship between residual hemipenis size and mean testis volume during the mating season and no relationship during the non-mating season (Fig. 3.5). Once phylogenetic effects had been removed from the analysis, there was still a significant positive relationship between residual

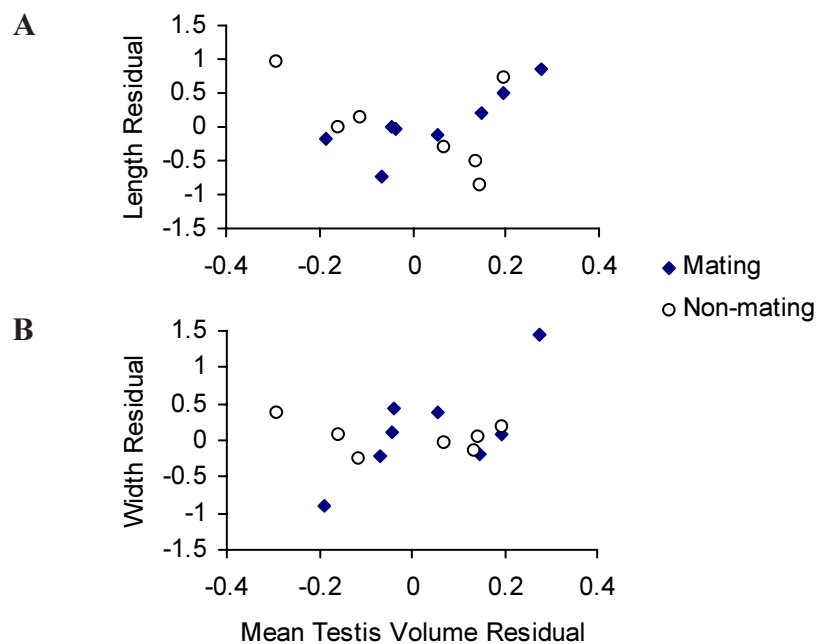


Fig. 3.5: Relationship between mean residual testis volume and hemipenis size across New Zealand gecko species during the mating and non-mating season: A. Residual right hemipenis length; B. Residual right hemipenis width.

hemipenis length and mean testis volume during the mating season ($F_{1,6} = 13.70$, $r^2 = 0.70$, $P = 0.01$). The relationship between residual hemipenis width and mean testis volume remained strong but was no longer significant ($F_{1,6} = 5.41$, $r^2 = 0.47$, $P = 0.06$).

b) Surface features of the hemipenis

General morphology:

The hemipenes of all species studied were very similar in overall morphology, and no variation was seen between organs prepared from fresh, ethanol-stored and formalin-fixed material. The organ is bifurcated, with the sulcus spermaticus branching into each lobe (Fig. 3.6A). The entire surface of the hemipenis is reticulate, covered with toothed calyces, except for a single fold located between the lobes of the organ which is smooth. On the asulcal surface, these calyces open out into cup-shaped structures (Fig. 3.6B). In *H. maculatus*, no seasonal change in overall hemipenis morphology was observed, suggesting that these surface features are retained throughout the year.

Size of calyces:

Both the overall width of the calyces and the height of the teeth were measured (Fig. 3.7). Neither of these measures were significantly related to SVL (Width: $F_{1,13} = 1.42$, $r^2 = 0.10$, $P = 0.25$; Height: $F_{1,13} = 3.27$, $r^2 = 0.20$, $P = 0.09$). There was great interspecific variation in the dimensions of calyces, with more than a two-fold difference in both width and height. In contrast, the measurement error due to tilting biases (see methods) was calculated to be much less than this (maximum estimate = 135%).

Analysis of covariance was used to determine whether there was a seasonal change in size of the calyces and whether size was related to mating system. There was a significant seasonal effect for width ($F_{1,9} = 15.83$, $P = 0.003$), with dimensions being greater during the non-mating season. After controlling for phylogeny this effect was no longer significant ($t_3 = -2.52$, $P > 0.05$). However, since all contrasts were negative, meaning that there was a trend for the width of calyces to be smaller during the mating season, this lack of significance is most likely to be due to the small sample size. There was no change in height of the teeth during the year (Height (\log_{10} transformed): $F_{1,9} = 2.81$, $P = 0.13$). There was no relationship between either dimension and relative testis volume (Width: $F_{1,9} = 0.13$, $P = 0.73$; \log_{10} Height: $F_{1,9} = 0.13$, $P = 0.72$) and no

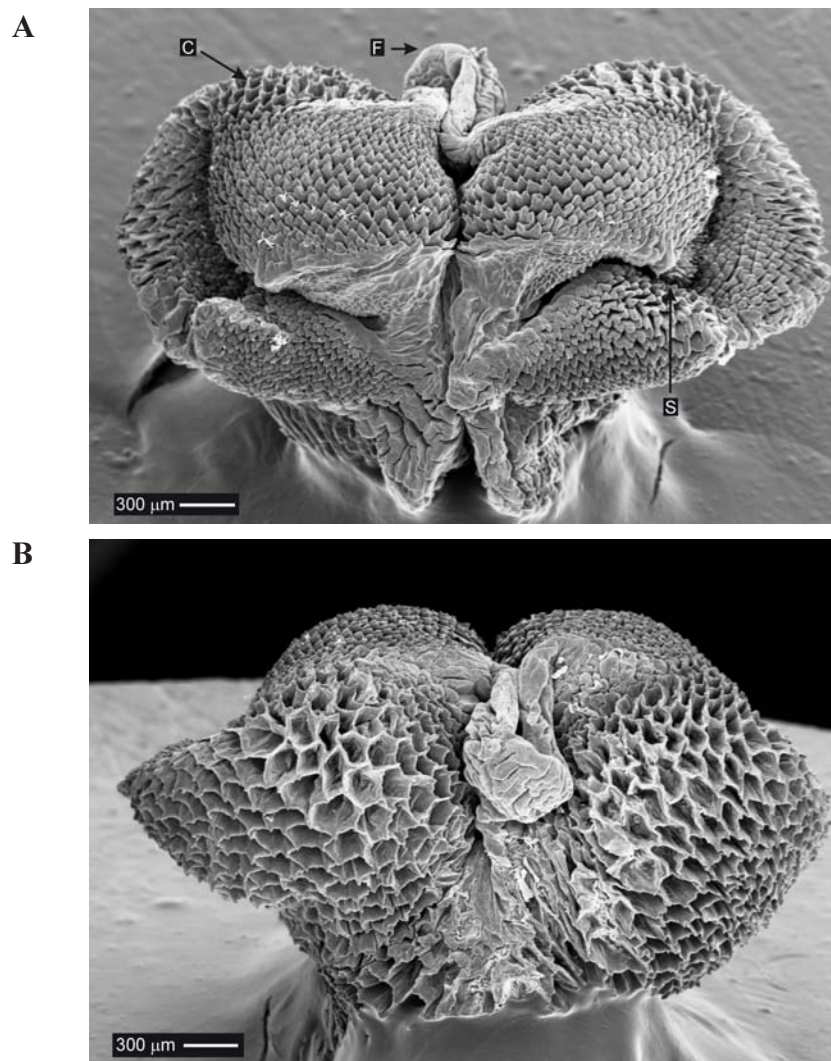


Fig. 3.6: SEM micrographs of hemipenis of *Hoplodactylus maculatus*: A. Sulcal surface showing sulcus spermaticus (S), calyces (C) and fold (F); B. Asulcal surface showing calyces opened out into cup-shaped structures.

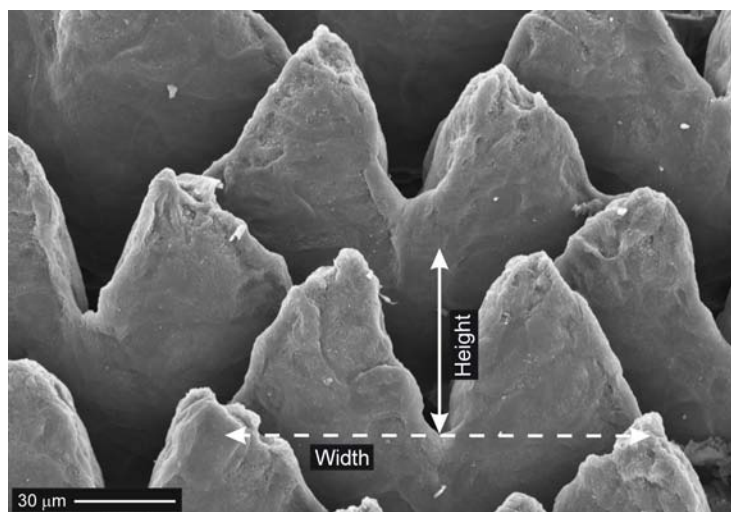


Fig. 3.7: SEM micrograph of calyces covering surface of hemipenis showing location of height and width measurements.

interaction between season and relative testis volume (Width: $F_{1,9} = 3.30$, $P = 0.10$; \log_{10} Height: $F_{1,9} = 1.46$, $P = 0.26$).

Density of calyces:

The density of calyces covering the hemipenis was not related to SVL (\log_{10} transformed data: $F_{1,12} = 0.53$, $r^2 = 0.04$, $P = 0.48$). There was nearly a four-fold difference in density of calyces between species, varying from 107.34/mm² in *H. maculatus* “Three Kings” to 411.12/mm² in *H. pacificus*. Analysis of covariance (\log_{10} transformed data) was again used to determine both whether there was a seasonal change in density and whether the density of calyces was related to mating system. There was no difference in density between the mating and non-mating season ($F_{1,8} = 0.30$, $P = 0.60$). There was also no relationship between density and mean testis volume residual ($F_{1,8} = 0.27$, $P = 0.62$) and no interaction between season and mean testis volume residual ($F_{1,8} = 0.02$, $P = 0.91$), indicating that the density of calyces was not related to mating system across species.

Sperm Morphology

a) Gross morphology

All species of New Zealand gecko analysed had filiform sperm. There was some variation between species in the orientation of the head: in the majority of species the head lay in the same plane as the midpiece and tail (Fig. 3.8A); however, in some individuals of *H. maculatus* “Canterbury” some sperm were observed with their heads bending back on themselves (Fig. 3.8B).

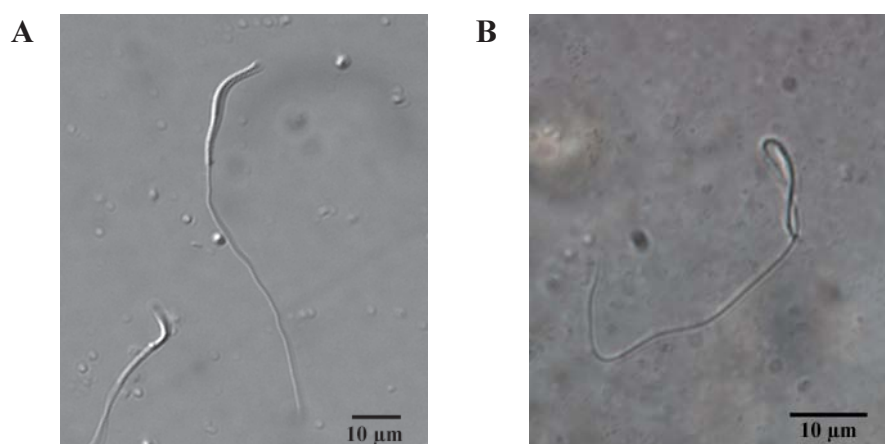


Fig. 3.8: Sperm from two species of New Zealand gecko showing variation in the orientation of the head: A. *H. pacificus* with the head and tail in the same plane; B. *H. maculatus* “Canterbury” with the head bent back. Photographs taken using a Zeiss Axioskop 2 MOT epifluorescent microscope fitted with a Zeiss AxioCam HRc CCD camera, using Zeiss AxioVision 3.1 software.

b) Intraspecific variation in sperm length

Two discrete sperm morphs were identified in samples from *H. maculatus* “Canterbury”: long, fertilising sperm (mean length = $70.85 \pm 0.72 \mu\text{m}$) and short, non-fertilising sperm (mean length = $30.11 \pm 1.45 \mu\text{m}$). The morphology and potential function of these are discussed in Chapter 6. In this study, only the long fertilising sperm were measured and compared across species

There was a significant negative relationship between total sperm length and SVL in *H. maculatus* “Canterbury” ($F_{1,6} = 10.69$, $r^2 = 0.64$, $P = 0.02$). The reason for this is unclear and could simply be due to the small sample size. There was no significant difference in sperm length between males for either raw lengths or residual values (raw: $F_{7,39} = 0.64$, $P = 0.72$; residual: $F_{7,39} = 0.17$, $P = 0.99$).

c) Interspecific variation in sperm length

There was no significant relationship between sperm length and SVL across species for either fresh ($F_{1,2} = 0.91$, $P = 0.44$) or fixed ($F_{1,16} = 0.57$, $P = 0.46$) sperm. Consequently, raw length data was used in all analyses.

I found that the total sperm length estimates made from fixed sperm fell close to or within the observed range for fresh sperm for *H. maculatus* “Canterbury” (fixed estimate: $55 \mu\text{m}$; fresh range: $56.73\text{--}81.74 \mu\text{m}$), *N. grayii* (fixed: $32.5 \mu\text{m}$; fresh: $38.56\text{--}75.99 \mu\text{m}$) and *N. stellatus* (fixed: $50.55 \mu\text{m}$; fresh: $36.42\text{--}57.34 \mu\text{m}$). However, fixed sperm measurements for *H. pacificus* were considerably smaller than those made from fresh sperm (fixed: $52.92 \mu\text{m}$; fresh: $87.81\text{--}92.49 \mu\text{m}$). This indicates that estimates made from fixed sperm are relatively reliable, but should be viewed with some caution, as for some species sperm breakage and/or shrinkage could have been significant.

There was a more than two-fold difference in sperm length between species (range: $32.5 \mu\text{m}$ (*N. grayii*) - $77.5 \mu\text{m}$ (*H. maculatus*)). There was no relationship between total sperm length and residual testis volume for fixed sperm ($F_{1,16} = 0.04$, $P = 0.85$). Unfortunately, there was an insufficient number of species to test this relationship using fresh sperm samples. However, values for these four species fell within the range for fixed sperm (Fig. 3.9).

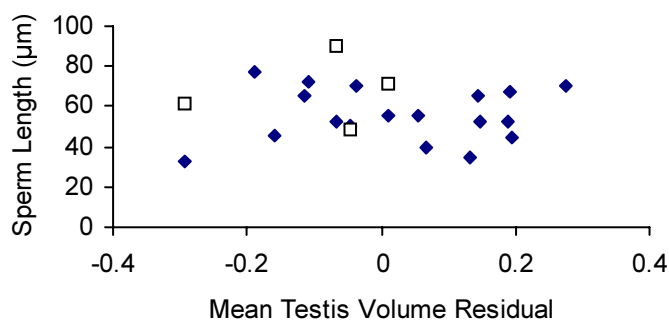


Fig. 3.9: Relationship between mean testis volume residual and both fixed (closed symbols) and fresh (open symbols) sperm length in New Zealand geckos.

Discussion

Hemipenis Morphology

There was a large amount of intraspecific variation in both length and width of the hemipenis between *Hoplodactylus maculatus* males. This variation could not be explained by differences in body size and was not related to mating season, contrary to findings in several other lizard groups (Arnold, 1986a; Branch, 1982; Glaw et al., 1999). This suggests that some other, unmeasured factor influences hemipenis size in this species. Large amounts of intraspecific variation in hemipenis size have also been observed in snakes (Zaher and Prudente, 1999), although it has been argued that much of this variation could be due to the preparation technique (Dowling, 2002). Such variation could have important implications for male reproductive success, as it might be expected that males with longer hemipenes would be able to place their sperm deeper within the female reproductive tract and thus gain an advantage when in competition with the ejaculates of other males (Eberhard, 1985). For example, in the water strider, *Gerris lateralis*, Arnqvist and Danielsson (1999) showed that the shape of the male intromittent organ significantly affected the degree of sperm precedence and thus fertilisation success.

Hemipenis size also varied between species. There was a significant positive correlation between hemipenis size and testis volume residual for both fresh everted hemipenes (length only) and preserved hemipenes (length and width during the mating season). This suggests that species with a higher intensity of sperm competition have evolved larger hemipenes. Similar relationships between intromittent organ size and testis size have been seen in birds (Coker et al., 2002) and mammals (Brownell and Ralls, 1986; Dixon, 1987; Miller et al., 1998). Larger intromittent organs may confer

two competitive advantages on males. Firstly, it may allow them to position their sperm more favourably within the female reproductive tract, giving them a competitive advantage over other males (Eberhard, 1985). Alternatively, it could allow males to access deep storage sites and remove the sperm of previous rival males (Waage, 1979). Since hemipenis morphology is also likely to be influenced by female reproductive tract morphology, the relationship between hemipenis size and oviduct length needs to be analysed to determine the precise mechanism causing this interspecific variation. In addition, some caution is required in interpreting the significance of the observed relationship between hemipenis size and testis size in New Zealand geckos, as the magnitude of interspecific variation was no greater than that observed within *H. maculatus*, suggesting that across species hemipenis size is relatively uniform.

Surface features of the hemipenes are highly conservative across species of New Zealand gecko. All species have bilobed hemipenes covered with toothed calyces. These calyces may serve similar functions to spines and bristles observed in other taxa, acting as scraping devices and enabling the removal of rival males' sperm (Danielsson, 1998; Eberhard, 1985; Gage, 1992; Waage, 1979). In addition, it has been suggested that the open cup-shaped structures formed by the interlocking calyces may have a suctional function (Cope, 1894, 1895), which may also aid sperm removal. No sperm were evident on the specimens I examined. However, this is not surprising as the fixation and preparation techniques used would have dislodged any sperm that may have been present.

There was no seasonal variation in the size of calyces in *H. maculatus*, unlike in other lizard species (Arnold, 1986a; Branch, 1982; Glaw et al., 1999). There was, however, seasonal variation in the width of calyces across species. A reduction in width during the mating season would be expected if there was also a concurrent increase in density, potentially increasing the surface area over which the scraping or suctional sperm removal mechanisms could operate. However, no seasonal change in density was observed.

There was large interspecific variation in both the dimensions and density of calyces. However, none of these measures were related to relative testis volume and thus predicted sperm competition intensity. This was unexpected as, if calyces do function as sperm removal devices, it would be expected that species with greater levels of sperm competition would have evolved to have a greater density of calyces, possibly with a

concurrent reduction in size. Such a relationship between intromittent organ surface features and mating system or testis size has been observed in other taxa (Coker et al., 2002; Dixon, 1987). Where this relationship does not exist it may suggest that surface features serve some purpose other than aiding in sperm competition (Harcourt and Gardiner, 1994). Olsson and Madsen (1998) showed that, in general, lizards have less spiny hemipenes and smaller testes than snakes, potentially reflecting the shorter duration of copulation in the former group. Compared with many other lizards and snakes, where hemipenis ornamentation includes features such as bristles, spines and hairs (Arnold, 1986a; Branch, 1982; Cope, 1894, 1895), the New Zealand geckos have relatively simple hemipenis morphology with little variation between species, suggesting that hemipenis morphology is not under strong selection pressure in this group.

Sperm Morphology

There was a negative relationship between sperm length and body size (SVL) in *H. maculatus* “Canterbury”. This was an unusual finding, as in other taxa there is generally either no relationship (e.g. Breed and Taylor, 2000; Briskie and Montgomerie, 1992; Gomendio and Roldan, 1991; LaMunyon and Ward, 2002; Morrow and Gage, 2000; Presgraves et al., 1999) or a positive relationship (e.g. Gage, 1994; Pitnick, 1996) between these two traits. Since no such relationship was observed in the cross-species comparison, it is likely that this result is simply due to the small sample size. However, it would be interesting to increase the sample size in the future to determine whether this is the case.

There was little intraspecific variation in sperm length in *H. maculatus* “Canterbury”. In contrast, there was more than a two-fold difference between species. Although this difference is relatively small when compared with that observed in other taxa (up to six times in birds [Briskie and Montgomerie, 1992; Briskie et al., 1997] and 60-100-fold variation in insects [Morrow and Gage, 2000; Presgraves et al., 1999]), it is still greater than the degree of intraspecific variation, suggesting that some selection pressure has been operating on sperm length in this group.

There was no relationship between the length of fixed sperm and relative testis size across species. It was initially thought that this could be due to fixed sperm estimates being inaccurate due to sperm breakage. However, the fact that fixed sperm measurements generally fell close to those for fresh sperm suggests that this lack of a

relationship is probably real. This finding contrasts markedly with the situation observed in many other taxa, where there is a positive relationship between sperm length and sperm competition intensity (Balshine et al., 2001; Briskie et al., 1997; Gomendio and Roldan, 1991; LaMunyon and Ward, 1999; although see Stockley et al. (1997) for a negative relationship in some fish). However, a similar lack of correlation between sperm length and relative testis size has been found in some birds (Briskie and Montgomerie, 1992) and mammals (Gage and Freckleton, 2003). This may suggest that testis size is too crude an estimate of sperm competition intensity in some cases. However, this seems unlikely as there is a strong relationship between relative testis size and mating system in many other taxa (Chapter 2) and several other studies have demonstrated a positive relationship between testis size and sperm length (Balshine et al., 2001; Breed and Taylor, 2000; Johnson and Briskie, 1999; Morrow and Gage, 2000). Therefore, it is more probable that this variation in sperm length in New Zealand geckos is the result of some alternative evolutionary pressure. It has been suggested that the observed correlation between sperm length and mating system in some birds may be due to an indirect relationship, as most variation in sperm length is explained by variation in sperm storage site size which, in turn, is correlated with mating system (Briskie et al., 1997). This possibility is investigated in Chapter 4.

Conclusions

Hemipenis morphology of New Zealand geckos appears to be highly conservative, with very little diversification between species. The fact that there is such large variation in other male reproductive characteristics, such as relative testis volume and sperm length, reflects the fact that there is a large amount of sexual selection in this group. Thus the evolution of hemipenis morphology appears to have been constrained for some reason. Hemipenis size is correlated with relative testis volume across species, suggesting that species with more intense sperm competition have larger hemipenes. This may enable them to position their sperm more favourably within the female reproductive tract or to access regions in which previous males have deposited their sperm and remove these. The latter explanation lacks support from the data, however, as there is no relationship between surface features of the hemipenis and relative testis volume. Sperm length does not vary with sperm competition intensity, suggesting that some other evolutionary pressure has brought about the observed variation.

Chapter 4: Female Reproductive Morphology and Sperm Competition

Introduction

In species with sperm competition, there is competition between male ejaculates for fertilisation of the female's ova. Thus selection acts upon male reproductive features (Chapter 3). However, the female can also have considerable influence over the outcome of sperm competition via modifications of her own reproductive tract. Eberhard (1985) recognised that females often have particularly long reproductive tracts and proposed that these may serve to prevent males from depositing sperm directly upon the eggs. This observation was then expanded to develop the theory of 'cryptic female choice' (Eberhard, 1996), where it was argued that females may be able to influence which sperm fertilise their eggs by processes such as biased transport of sperm or selective utilisation of sperm within the reproductive tract. However, perhaps the modification that is likely to give females the greatest control over which male ultimately fertilises her ova is the development of sperm storage sites.

The ability to store sperm has been demonstrated in all vertebrate classes with internal fertilisation except the jawless fish (Class Agnatha) (see review by Howarth, 1974). In the majority of cases, sperm are stored in specialised regions. The duration of sperm storage is highly variable between groups, being shortest in mammals, where it is typically less than 24 h, and longest in reptiles, where sperm may be stored for up to seven years (Birkhead and Møller, 1993). It is believed that this variation may be partly due to the temperature at which sperm are maintained (Birkhead and Møller, 1993; Howarth, 1974): mammals and birds, being endotherms, tend to maintain their bodies at greater temperatures on average than the ectothermic reptiles; these higher temperatures are believed to shorten the life span of sperm.

In reptiles, sperm storage sites are commonly found in the vagina (most frequently near the junction with the uterus) and/or in the uterine tube or infundibulum (e.g. Cuellar, 1966b; Fox, 1963; Halpert et al., 1982; also see reviews: Girling, 2002; Gist and Jones, 1987; Sever and Hamlett, 2002). The exact location can vary even within members of the same family, as exemplified by the gekkonid lizards (e.g. Girling et al., 1997, 1998; King, 1977; MacAvoy, 1976; Murphy-Walker and Haley, 1996; Whittier et al., 1994).

Furthermore, it has been shown that the region used for sperm storage can vary according to the stage of the breeding cycle. For example, in *Hoplodactylus maculatus*, Girling et al. (1997) found that sperm were stored in the vagina over winter and then moved up to the infundibular storage sites early in spring, ready for ovulation.

In most species of reptile, sperm storage sites are located at the base of mucosal folds which run longitudinally along the oviduct. In some cases these regions are tubular pockets formed from the epithelium (Adams and Cooper, 1988; Conner and Crews, 1980; Cuellar, 1966b; King, 1977; Shanthakumari et al., 1990). In other species, however, including species of New Zealand geckos examined to date, glands are used as sperm storage sites (Aldridge, 1992; Girling et al., 1997, 1998; MacAvoy, 1976; Sever et al., 2000). Sperm are often oriented within the storage regions with their heads directed toward the distal end (Conner and Crews, 1980; Fox, 1963; Gist and Jones, 1989; Halpert et al., 1982; Hattan and Gist, 1975); in some instances, the heads are seen buried into the epithelial lining of the glands (Adams and Cooper, 1988; Cuellar, 1966b; Sever and Hamlett, 2002). It has been proposed that this may represent a mechanism by which sperm obtain nutrients from the surrounding cells (Cuellar, 1966b). Other suggested methods for the long-term maintenance of sperm within storage sites include nutrition provided in the form of glycoprotein granules, either inseminated with the sperm (Srinivas et al., 1995) or secreted from the epithelium of the storage site (Cuellar, 1966b; Halpert et al., 1982), and suppression of metabolism and/or activity of the sperm (Gist and Jones, 1987). Prior to, or at the time of, ovulation, sperm need to leave these storage sites and travel up the oviduct to the fertilisation site, which is likely to be in the infundibulum. It has been proposed that this evacuation of sperm could be achieved through stimulation of the muscles in the walls of the storage sites, which are continuous with the muscle bands that extend throughout the oviduct, at the time of ovulation (Cuellar, 1966b; Fox, 1963; King, 1977).

The duration of sperm storage in reptiles is highly variable, ranging from months to years (Gist and Jones, 1987). However, evidence for very long-term storage is often weak, as it is not conclusively shown whether young are produced via fertilisation of ova from stored sperm or parthenogenesis (Fox, 1977; Mangusson, 1979). Nevertheless, there is strong evidence that sperm are stored in many species for at least several months, as in many temperate species, including New Zealand geckos, male and female

reproductive cycles are asynchronous (e.g. Girling et al., 1997; King, 1977; MacAvoy, 1976; Smyth and Smith, 1968; Srinivas et al., 1995). This means that the mating season is temporally separated from the ovulatory period, so that sperm storage is obligatory. The temporal separation of these events may allow females inhabiting temperate climates to ovulate earlier in the spring than would be possible were they reliant upon temperatures suitable for mating activity; this in turn results in the young hatching or being born earlier and thus having a longer period during which they can feed and grow before their first winter (Smyth and Smith, 1968). However, since sperm storage is also often found in species where the sexes have synchronous reproductive cycles, there must be additional advantages to the female in storing and maintaining sperm. One possible advantage is that sperm storage may ensure that females can fertilise multiple clutches of eggs, including those laid at the end of the mating season, allowing their reproductive cycle to extend beyond that of males (Cuellar, 1966a; Shanthakumari et al., 1990). It is also possible that females may gain some genetic advantage by mating with more than one male (Devine, 1984). There is evidence that female reptiles that mate with multiple partners may gain benefits in terms of hatching success, offspring viability and juvenile survivorship (Madsen et al., 1992; Olsson et al., 1994b; Olsson et al., 1994c; Olsson and Shine, 1997). Thus, sperm competition may have been an important selection pressure in the evolution of sperm storage sites.

The relationship between sperm storage site morphology and sperm competition intensity has been best studied in birds where there is often no direct relationship between the two (Briskie, 1993; Briskie and Montgomerie, 1993; Briskie et al., 1997). However, a large amount of interspecific variation in storage site size and number does exist, and this is often related to characteristics of the male ejaculate, including sperm number and length (Birkhead and Møller, 1992; Briskie and Montgomerie, 1992, 1993; Briskie et al., 1997). It has been suggested that sperm competition may result in direct selection on sperm length and number, which in turn creates a selection pressure on sperm storage site morphology (Briskie and Montgomerie, 1992). Thus the observed variation in sperm storage site number and size may be due to indirect selection from sperm competition.

It has previously been demonstrated that females of several species of New Zealand gecko have sperm storage sites (Girling et al., 1997, 1998; MacAvoy, 1976). Therefore, in this chapter I make a cross-species comparison of sperm storage site morphology in

New Zealand geckos and relate number and size of the sites to both sperm competition intensity and sperm length. I predict that species experiencing greater intensities of sperm competition will have fewer and/or longer SSTs and an associated increase in sperm length, as previously found in birds (Briskie and Montgomerie, 1993; Briskie et al., 1997).

Methods

To determine intraspecific variation and seasonal variation in oviduct morphology, the left oviduct was removed from 16 female *Hoplodactylus maculatus*, collected from Turakirae Head by A.H. Whitaker (see Whitaker (1982) for a description of the study site and collection techniques). Individuals collected between February and August (1967-68) were used, as this is the period of sperm storage in this species (Robinson, 1985). In addition, females outside this period were pregnant, making observations of oviduct morphology particularly difficult. These individuals were placed directly in ethanol upon capture, with no prior fixation. To investigate interspecific variation in oviduct morphology, the left oviduct was removed from a further 19 individuals from 16 species (*H. duvaucelii*, *H. granulatus*, *H. maculatus*, *H. maculatus* “Canterbury”, *H. maculatus* “Eastern Otago”, *H. maculatus* “Marlborough”, *H. maculatus* “Poor Knights”, *H. maculatus* “Southern Alps”, *H. maculatus* “Three Kings”, *H. pacificus*, *Naultinus elegans elegans*, *N. e. punctatus*, *N. gemmeus*, *N. grayii*, *N. manukanus* and *N. rudis*). These specimens were provided by the Museum of New Zealand Te Papa Tongarewa and from individuals that died during behavioural studies (Chapter 5), and had all been fixed in formalin and stored in 70% ethanol. Where possible individuals collected during the sperm storage period (defined as the period from mating to ovulation (see Appendix A for dates)) were used. However, such specimens were not available for all species. In addition, it should be noted that dates provided by museums may have been accession dates rather than collection dates for certain specimens (Cree A, pers. comm.).

Whole-Mount Observation of Morphology

Each oviduct was dissected under a Leica MZ6 stereomicroscope and its morphology investigated following the methodology of Briskie and Birkhead (1993). A longitudinal incision was made along each oviduct, and the organ was pinned flat on a cork dissecting

board and covered with distilled water. The oviduct was separated into three separate regions for the purposes of this study: vagina, uterus and uterine tube (Fig. 4.1). There were a number of discrete folds evident in both the vagina and uterine tube in most individuals. The total number of folds in each region was counted. Three folds were then removed from each region and opened out to lie flat upon a glass slide, using a microscalpel and fine forceps. Distilled water was added to the slide, and a coverslip placed over the top to keep moist and further flatten the tissue. Folds were then viewed unstained with a LEICA DMR light microscope at x400 magnification with phase-contrast illumination. Putative sperm storage tubules (hereafter referred to as SSTs) could be identified as discrete round-oval structures. The total number of tubules was counted for each fold. In order to estimate the total number of tubules in a region, the average number of tubules per fold was multiplied by the total number of folds observed. In addition, the length and width of up to 10 randomly selected tubules were measured using an ocular micrometer to make an estimate of average size (length and area (length x width)).

In the instance that folds were not obvious in a region, 1-3 sections of tissue were removed from the area. In the uterus these tissue sections were evenly spread, coming from the vagina end, middle and uterine tube end. In the case of the vagina or uterine tube, each section ran the length of the region. The total number of tubules on each tissue section was then counted, and an average calculated. In order to use these samples

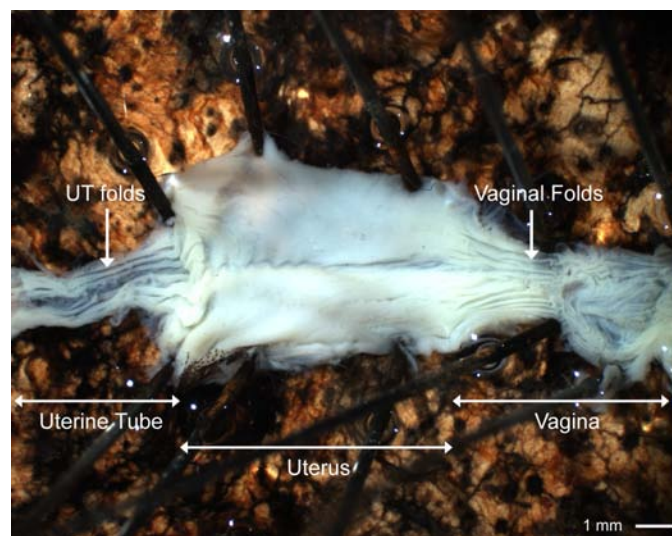


Fig. 4.1: Longitudinal dissection of oviduct of *Nautinus elegans punctatus*, showing three regions (vagina, uterus and uterine tube) and the location of folds in the vagina and uterine tube.

to estimate the total number of tubules in the area, the average number of tubules was multiplied by the total area of the region divided by the average area of tissue sample collected. The length and width of up to 10 randomly selected tubules was measured on each tissue sample to obtain an estimate of size.

Histological Examination of Morphology

Each fold or tissue sample used to estimate sperm storage site number and size for each region of the oviduct was sectioned and stained to confirm the presence of tubules and determine whether they contained sperm.

All tissue was stored in 70% ethanol. Since samples were very small, pre-staining of the white tissue was required for improved visibility during processing. Samples were rehydrated for 10 min in 50% ethanol and distilled water and then pre-stained in double strength Mayer's haemalum for 5 min and placed in tap water for 5 min to blue. Following this, samples were dehydrated through an ascending concentration gradient of ethanol, for 10 min at each concentration (50%, 70%, 90%, 100%, 100%). The tissue was then cleared in toluene for 10 min and infiltrated with paraplast wax by placing it under vacuum twice. Samples were cast into small wax blocks and 5 μ m sections were cut with a microtome. Sections were mounted on slides, dewaxed and stained using Mayer's double-strength haemalum and eosin.

Slides were viewed at x400 magnification. Presence/absence of SSTs (as identified using the whole-mount technique) was recorded for each sample. In addition, each tubule was examined for the presence of sperm.

Data Analysis

Since there is considerable variation in body size both within and between species of New Zealand gecko, each variable was first regressed against snout-vent length (SVL). In the cross-species comparison, weighted regression analysis was used to control for the increased influence of *H. duvaucelii*, a major outlier. Where there was a significant relationship with SVL, residual values were used in analyses to control for body size effects.

Intraspecific variation in sperm storage site morphology was used to investigate whether oviduct morphology varied seasonally. To determine whether the intensity of sperm competition has influenced female reproductive tract morphology, across species

sperm storage site morphology was correlated with relative testis volume, as calculated from the relationship between mean testis volume and SVL for the New Zealand geckos (see Chapter 2). In addition, the relationship between SST morphology and sperm length was investigated. In all analyses, residuals were checked for normality and homogeneity of variance; data transformations were applied where necessary.

The ‘contrast method’ (Purvis and Rambaut, 1994) was used to control for phylogeny in all cross-species comparisons for which a significant relationship was detected, as outlined in Chapter 2. I used a phylogeny constructed by Hitchmough (1997), generated from allozyme data and using Nei’s D method, and assumed that all branch lengths were equal. Populations of the *H. maculatus* species complex arising from different geographic locations were treated as separate species. Where necessary, data were transformed to meet evolutionary and statistical assumptions of this analysis.

Photographs were taken using a Leica MZ12s stereomicroscope and a Zeiss Axioskop 2 MOT epifluorescent microscope fitted with a Zeiss AxioCam HRC CCD camera and using Zeiss AxioVision 3.1 software.

Results

Oviduct Morphology in New Zealand Geckos

Histological sectioning and staining of folds revealed SSTs of similar structure to those observed using whole-mount techniques (Fig. 4.2). Unfortunately, sperm could not be seen within any of the tubules, either under whole mount or in the tissue sections. Cells lining the tubules were identical to those found lining the oviduct (Fig. 4.3), suggesting that these regions are invaginations formed within the wall of the oviduct.

A presence/absence comparison of results from whole-mount with those from histological sectioning revealed that overall the whole-mount method was fairly reliable in detecting the presence of SSTs. Occasionally SSTs were found within stained sections that had not been noticed previously: this was especially common in the uterus, where deep pigmentation, particularly around the time of ovulation and during pregnancy, made observation of SSTs very difficult. In addition, sectioning did not always reveal SSTs where they had been detected using whole-mount techniques, largely due to poor preparation of the tissue for certain samples. Since results from the whole-mount technique were used for all analyses, any bias in the results should be fairly consistent between samples.

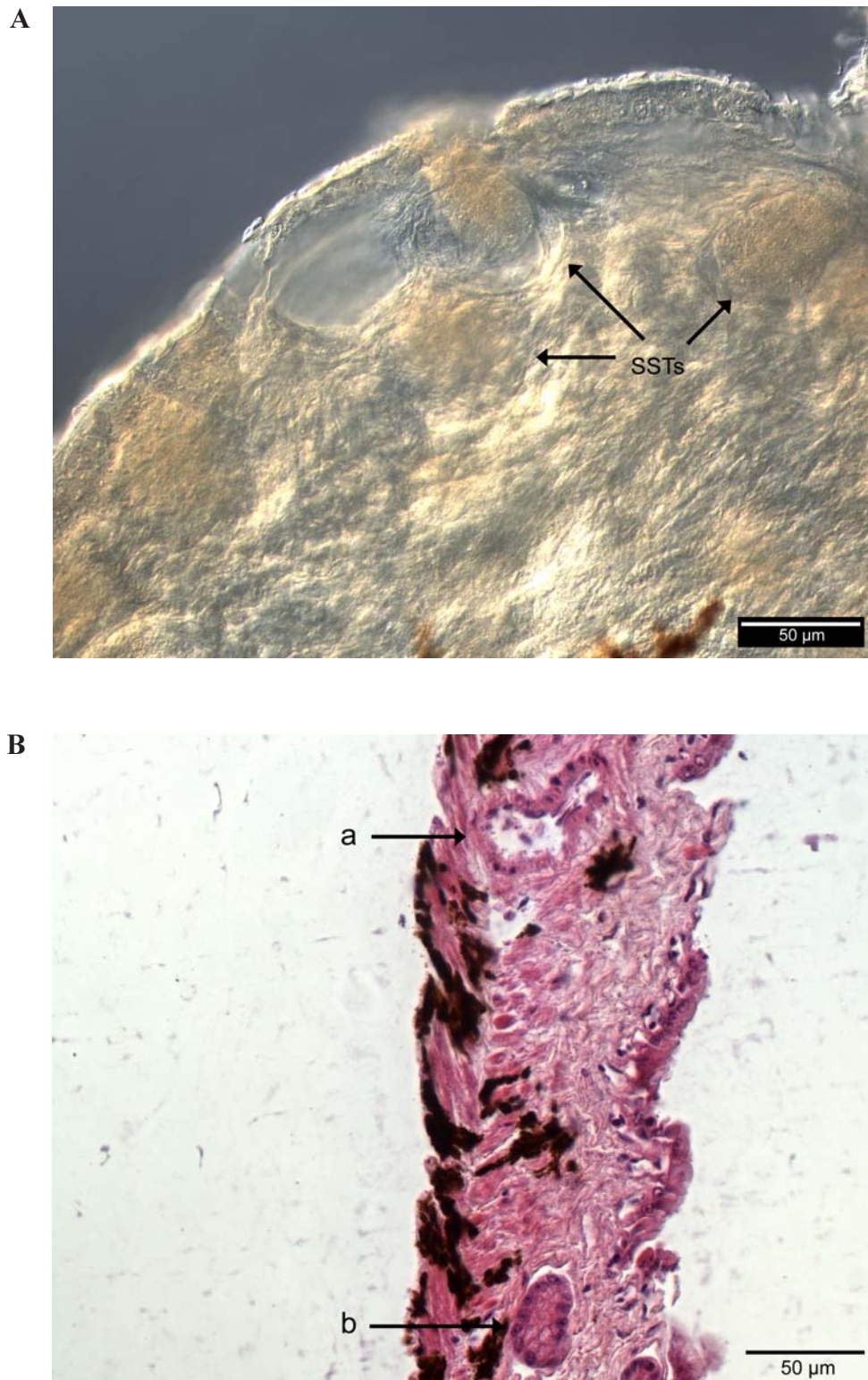


Fig. 4.2: SSTs in the uterine tube of *Hoplodactylus maculatus*: A. Whole-mount, unstained section; B. Histological section stained with Mayer's double-strength haemalum and eosin showing SST with lumen containing debris (a) and SST without lumen (b).



Fig. 4.3: Transverse section of the vagina of *Hoplodactylus maculatus* stained with Mayer's double-strength haemalum showing folded epithelium.

Seasonal Variation in Sperm Storage Site Morphology in H. maculatus

There was no significant relationship between SVL and the number of folds (Vagina (rank data): $F_{1,14} = 4.18$, $r^2 = 0.23$, $P = 0.06$; Uterine Tube: $F_{1,14} = 0.57$, $r^2 = 0.04$, $P = 0.46$) or the number of SSTs (Vagina (\log_{10} transformed): $F_{1,14} = 1.10$, $r^2 = 0.07$, $P = 0.31$; Uterine Tube (sqrt transformed): $F_{1,13} = 0.60$, $r^2 = 0.04$, $P = 0.45$; Uterus (sqrt transformed): $F_{1,12} = 0.07$, $r^2 = 0.006$, $P = 0.80$; Total: $F_{1,14} = 1.37$, $r^2 = 0.09$, $P = 0.26$) in any region of the oviduct. There was no relationship between SVL and the size of SSTs in either the vagina (Length (sqrt transformed): $F_{1,8} = 0.76$, $r^2 = 0.09$, $P = 0.41$; Area: $F_{1,8} = 1.52$, $r^2 = 0.16$, $P = 0.25$) or uterine tube (Length (rank data): $F_{1,11} = 0.47$, $r^2 = 0.04$, $P = 0.51$; Area: $F_{1,11} = 0.61$, $r^2 = 0.05$, $P = 0.45$). There was also no association between the length of SSTs in the uterus and SVL ($F_{1,10} = 2.63$, $r^2 = 0.21$, $P = 0.14$). There was, however, a significant negative relationship between the area of SSTs in the uterus and SVL ($F_{1,10} = 5.14$, $r^2 = 0.34$, $P = 0.047$). Therefore, residual values were calculated for this variable and used in all analyses.

There was considerable intraspecific variation in the number of folds and the number and size of SSTs in all regions of the oviduct (Table 4.1). There was no significant difference between the number of folds located in the vagina and uterine tube (paired t-test: $t_{15} = 1.96$, $P = 0.07$). In contrast, there was a significant difference between the

Table 4.1: Intraspecific variation in the number of folds and number and size of SSTs in each region of the oviduct in *Hoplostactylus maculatus*. Values presented are mean \pm SE; range provided in parentheses. Range of residual values (corrected for SVL) presented for uterus area [absolute values they represent beneath].

Region	No. folds	No. SSTs	Size of SSTs	
			Length (µm)	Area (µm ²)
Vagina	10.63 \pm 0.88 (0 - 14)	18.04 \pm 5.54 (0 - 70)	63.82 \pm 8.08 (29.2 - 125)	2940.40 \pm 623.39 (589.58 - 7750)
Uterine Tube	7.38 \pm 1.10 (0 - 15)	462.39 \pm 117.19 (0 - 1660)	60.58 \pm 2.60 (38.75 - 76.08)	2655.17 \pm 233.83 (1036.88 - 4176.04)
Uterus	NA	568.84 \pm 131.70 (0 - 1454)	48.77 \pm 3.50 (28.75 - 76)	1819.75 \pm 265.95 (-1170.57 - 1232.79) [656.25 - 3941.88]
Total	NA	954.41 \pm 165.51 (14 - 1901)	NA	NA

number of SSTs located in each region (Blocked ANOVA (\log_{10} transformed data; female as block): $F_{2,27} = 9.61$, $P < 0.001$), with, on average, the uterus and uterine tube having more SSTs than the vagina (Tukey Test - Uterus x Vagina: $q_{24,3} = 5.95$, $P < 0.001$; Uterine Tube x Vagina: $q_{24,3} = 5.65$, $0.001 < P < 0.005$). There was also a significant difference in the size of SSTs between regions (Blocked ANOVA (both variables \log_{10} transformed; female as block) - Length: $F_{2,17} = 8.59$, $P = 0.003$; Area: $F_{2,17} = 7.57$, $P = 0.004$). SSTs in the vagina and uterine tube were longer than those located in the uterus (Tukey Test - Vagina x Uterus: $q_{17,3} = 3.65$, $0.025 < P < 0.05$; Uterine Tube x Uterus: $q_{17,3} = 4.49$, $0.01 < P < 0.025$) and SSTs in the uterine tube were significantly greater in area than those in the the uterus (Tukey Test - $q_{17,3} = 4.51$, $0.01 < P < 0.025$).

There was no significant variation between months in the number of folds (Vagina: $F_{5,10} = 1.40$, $P = 0.30$; Uterine Tube: $F_{5,10} = 1.21$, $P = 0.37$) or SSTs (Vagina: $F_{5,10} = 0.77$, $P = 0.59$; Uterine Tube (sqrt transformed): $F_{5,9} = 0.93$, $P = 0.51$; Uterus: $F_{5,8} = 1.22$, $P = 0.38$; Total: $F_{5,10} = 0.28$, $P = 0.92$). Similarly, there was no significant monthly variation in the size of SSTs in the vagina (\log_{10} transformed - Length: $F_{4,5} = 0.65$, $P = 0.65$; Area: $F_{4,5} = 0.58$, $P = 0.69$), uterine tube (Length: $F_{5,7} = 2.46$, $P = 0.14$; Area: $F_{5,7} = 3.49$, $P = 0.07$) or uterus (Length: $F_{5,6} = 2.59$, $P = 0.14$; Area: $F_{5,6} = 1.54$, $P = 0.31$).

When a comparison was made of sperm storage site morphology between the mating (February-May) and non-mating (June-August) season, it was found that there were significantly more folds in the vagina during the non-mating season ($t_{12} = -2.68$, $P = 0.02$). In addition, uterine tube SSTs tended to be longer during the non-mating season (mean: $66.25 \mu\text{m} \pm 2.90$) than the mating season (mean: $50.70 \mu\text{m} \pm 3.14$), although these differences were not quite significant ($t_{10} = -2.08$, $P = 0.06$).

Cross-Species Comparison of SST Morphology

There was no relationship between the number of folds in the vagina or uterine tube and SVL (Vagina (rank data): $F_{1,14} = 0.14$, $r^2 = 0.01$, $P = 0.71$; Uterine Tube: $F_{1,14} = 1.09$, $r^2 = 0.07$, $P = 0.32$). However, there was a significant positive relationship between SVL and the number of SSTs in all three regions and in total (Vagina: $F_{1,14} = 5.76$, $r^2 = 0.29$, $P = 0.03$; Uterine tube: $F_{1,14} = 4.86$, $r^2 = 0.26$, $P = 0.04$; Uterus (sqrt transformed): $F_{1,14} = 4.59$, $r^2 = 0.25$, $P = 0.05$; Total (number and SVL sqrt transformed): $F_{1,14} = 5.10$, $r^2 = 0.27$, $P = 0.04$). This relationship remained significant for all regions but the uterus after controlling for phylogeny (Vagina (log-log transformed data): $F_{1,11} = 10.81$, $r^2 = 0.50$, $P = 0.007$; Uterine Tube (SVL \log_{10} transformed): $F_{1,11} = 16.03$, $r^2 = 0.59$, $P = 0.002$; Total (log-log transformed data): $F_{1,11} = 14.00$, $r^2 = 0.56$, $P = 0.003$). Therefore, residual values were used for these variables in all analyses. Since the relationship between SVL and the number of SSTs in the uterus was approaching significance after controlling for phylogeny (log-log transformed data: $F_{1,11} = 4.25$, $r^2 = 0.28$, $P = 0.06$) and was significant using the raw data, residual values were also used for this variable in all analyses. The size of SSTs was not related to SVL in any region (Vagina: Length - $F_{1,11} = 0.02$, $r^2 = 0.002$, $P = 0.89$, Area - $F_{1,11} = 0.03$, $r^2 = 0.003$, $P = 0.86$; Uterine Tube: Length - $F_{1,14} = 0.30$, $r^2 = 0.02$, $P = 0.59$, Area - $F_{1,14} = 1.07$, $r^2 = 0.07$, $P = 0.32$; Uterus (rank data): Length - $F_{1,12} = 1.01$, $r^2 = 0.08$, $P = 0.33$, Area - $F_{1,12} = 0.76$, $r^2 = 0.06$, $P = 0.40$).

Across species there were significantly more folds in the vagina than the uterine tube (paired t-test: $t_{15} = 5.09$, $P < 0.001$). There were more SSTs in the uterine tube than vagina for all species and in 10 species the uterus had the most SSTs. There was a large amount of interspecific variation in SST morphology (Table 4.2). Maximum variation in number of SSTs was observed in the uterus (Range: 0-25592) and SSTs located in

Table 4.2: Interspecific variation in the number of folds and number and size of SSTs in each region of the oviduct in New Zealand geckos. Range of residual values (corrected for SVL) presented for number of tubules (corresponding absolute values in parentheses).

Region	No. folds	SSTs		
		Number	Length (i m)	Area (i m ²)
Vagina	8.7-16	-250.1 - 341.8 (3.3 - 526.7)	25 - 66.25	437.5 - 3169.38
Uterine Tube	0-12	-847.7 - 1290.2 (343.7 - 2224)	39.42 - 74.38	1142.71 - 3953.75
Uterus	NA	-46.6 - 60.6 (0 - 7459)	36.88 - 63.42	923.13 - 2987.92
Total	NA	-39.8 - 49.9 (346.44 - 7641.05)	NA	NA

the vagina were most variable in size (Range: Length = 25-66.25 μm ; Area = 437.5-3169.38 μm^2).

There was a significant positive correlation between SST number (corrected for body size) and both SST length and area in the vagina (Length: $r = 0.72$, $P = 0.005$; Area: $r = 0.66$, $P = 0.01$). This relationship remained significant after controlling for phylogeny (Length: $F_{1,8} = 53.08$, $r^2 = 0.87$, $P < 0.001$; Area: $F_{1,8} = 21.09$, $r^2 = 0.73$, $P = 0.002$). There was no correlation between SST number and size in any other region of the oviduct (Uterine Tube: Length - $r = 0.12$, $P = 0.67$, Area - $r = -0.09$, $P = 0.75$; Uterus: Length - $r = 0.43$, $P = 0.12$, Area - $r = 0.45$, $P = 0.11$).

a) SST morphology and relative testis size

Analysis of covariance was used to determine whether relative testis size was related to SST morphology and whether this relationship depended upon season (sperm storage vs non-sperm storage). There was no significant correlation between the number of folds or number or size of SSTs in any region of the oviduct and mean testis volume residual (used as a predictor of mating system) or season (Table 4.3). There was, however, a significant interaction between sperm storage season and relative testis volume for the number of folds in the vagina (Fig. 4.4). The number of folds decreased with

Table 4.3: Relationship between the number of folds and number and size of SSTs in each region of the oviduct, season (sperm storage vs non-sperm storage) and mean testis volume residual in New Zealand geckos. Asterisk (*) represents a significant effect ($p < 0.05$).

Region	Measure	Source	F	df	P
Vagina	No. folds	Season	2.87	1, 10	0.12
		Testis	0.70	1, 10	0.42
		Season x Testis	4.94	1, 10	0.05*
	No. SSTs	Season	0.12	1, 10	0.74
		Testis	0.19	1, 10	0.67
		Season x Testis	1.36	1, 10	0.27
	Length SSTs	Season	0.29	1, 7	0.61
		Testis	0.20	1, 7	0.66
		Season x Testis	1.78	1, 7	0.22
	Area SSTs	Season	0.35	1, 7	0.57
		Testis	0.22	1, 7	0.65
		Season x Testis	2.72	1, 7	0.14
Uterine Tube	No. folds	Season	0.11	1, 10	0.74
		Testis	2.22	1, 10	0.17
		Season x Testis	0.007	1, 10	0.94
	No. SSTs	Season	3.88	1, 10	0.08
		Testis	0.16	1, 10	0.69
		Season x Testis	0.07	1, 10	0.79
	Length SSTs	Season	0.05	1, 10	0.82
		Testis	0.28	1, 10	0.61
		Season x Testis	0.05	1, 10	0.82
	Area SSTs	Season	0.16	1, 10	0.69
		Testis	0.31	1, 10	0.59
		Season x Testis	1.22	1, 10	0.30
Uterus	No. SSTs	Season	1.25	1, 9	0.29
		Testis	2.08	1, 9	0.18
		Season x Testis	0.90	1, 9	0.37
	Length SSTs	Season	0.31	1, 8	0.59
		Testis	0.02	1, 8	0.89
		Season x Testis	3.05	1, 8	0.12
	Area SSTs	Season	0.09	1, 8	0.77
		Testis	0.18	1, 8	0.69
		Season x Testis	2.69	1, 8	0.14
Total	No. SSTs	Season	0.21	1, 10	0.66
		Testis	1.68	1, 10	0.22
		Season x Testis	1.15	1, 10	0.31

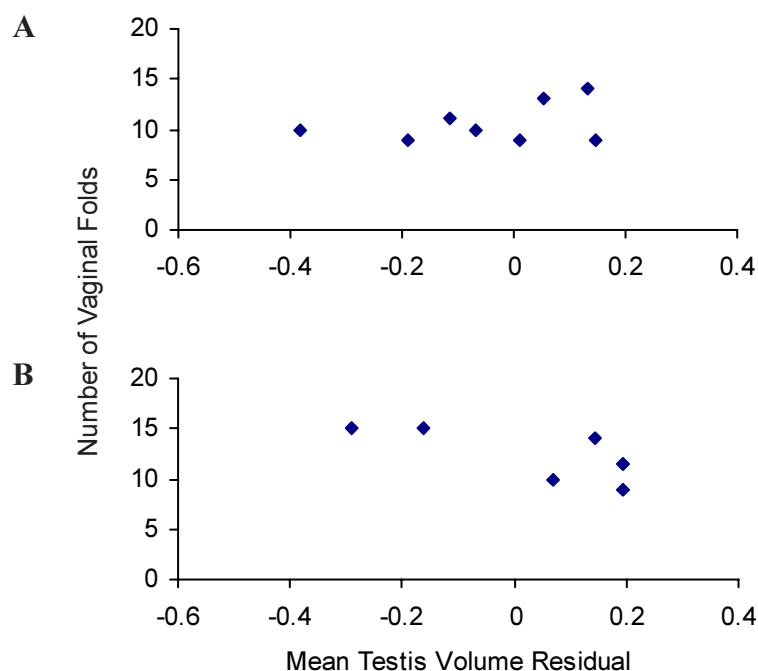


Fig. 4.4: Relationship between the number of folds in the vagina and mean relative testis volume in New Zealand geckos during: A. Sperm storage season; B. Non-sperm storage season.

increasing testis volume residual (and thus sperm competition intensity) during the non-mating season but remained relatively constant across species during the mating season. This relationship remained significant after controlling for phylogeny ($F_{1,3} = 18.95$, $r^2 = 0.86$, $P = 0.02$). No other interaction was significant.

b) SST morphology and sperm length

The number of folds and SSTs in both the vagina and uterine tube was not related to sperm length or season and there was no interaction between these variables (Table 4.4). There was, however, a significant negative correlation between sperm length and both the number of SSTs in the uterus and the total number of SSTs. This relationship became more significant after controlling for phylogeny (Uterus: $F_{1,9} = 17.44$, $r^2 = 0.66$, $P = 0.002$; Total: $F_{1,9} = 22.99$, $r^2 = 0.72$, $P = 0.001$). The statistical assumption of homogeneity of variance of residuals was violated in the phylogenetic control for the number of SSTs in the uterus and sperm length. However, since the result is equivalent to that obtained using the raw data it is probable that this relationship is real and not a result of inappropriate statistical analysis. The number of SSTs in the uterus and the total number did not vary with season and there was no interaction between season and

Table 4.4: Relationship between the number of folds and number and size of SSTs in each region of the oviduct, season (sperm storage vs non-sperm storage) and sperm length in New Zealand geckos. Asterisk (*) represents a significant effect ($p < 0.05$).

Region	Measure	Source	F	df	P
Vagina	No. folds	Season	1.80	1, 9	0.21
		Sperm	2.38	1, 9	0.16
		Season x Sperm	0.10	1, 9	0.75
	No. SSTs	Season	0.0004	1, 9	0.98
		Sperm	1.50	1, 9	0.25
		Season x Sperm	0.43	1, 9	0.53
	Length [†]	Season	0.56	1, 6	0.48
		Sperm	2.49	1, 6	0.17
		Season x Sperm	0.12	1, 6	0.74
	Area [†]	Season	0.62	1, 6	0.46
		Sperm	2.95	1, 6	0.14
		Season x Sperm	0.19	1, 6	0.68
Uterine Tube	No. folds	Season	0.08	1, 9	0.78
		Sperm	0.33	1, 9	0.58
		Season x Sperm	1.22	1, 9	0.30
	No. SSTs	Season	2.92	1, 9	0.12
		Sperm	0.01	1, 9	0.91
		Season x Sperm	0.12	1, 9	0.73
	Length	Season	0.13	1, 9	0.73
		Sperm	0.37	1, 9	0.56
		Season x Sperm	0.08	1, 9	0.78
	Area [†]	Season	0.03	1, 9	0.86
		Sperm	0.15	1, 9	0.71
		Season x Sperm	0.002	1, 9	0.96
Uterus	No. SSTs	Season	3.07	1, 8	0.12
		Sperm	7.69	1, 8	0.02 *
		Season x Sperm	2.70	1, 8	0.14
	Length	Season	0.06	1, 7	0.81
		Sperm	0.73	1, 7	0.42
		Season x Sperm	0.07	1, 7	0.79
	Area	Season	0.006	1, 7	0.94
		Sperm	0.53	1, 7	0.49
		Season x Sperm	0.21	1, 7	0.66
Total	No. SSTs	Season	0.64	1, 9	0.45
		Sperm	7.42	1, 9	0.02 *
		Season x Sperm	0.31	1, 9	0.59

[†] ANCOVA performed on log₁₀ transformed data

sperm length, meaning that this negative relationship remained throughout the year. The size of SSTs was not related to sperm length or season for any region of the oviduct and there was no interaction between these variables. A comparison of SST length and sperm length showed that it was fairly common for sperm to be longer than SSTs (% species - Vagina: 58%; Uterine Tube: 50%; Uterus: 83%).

Discussion

The traditional method used to analyse SST morphology is histological sectioning of the oviduct (Adams and Cooper, 1988; Cuellar, 1966b; Fox, 1963; King, 1977; MacAvoy, 1976; Sever and Hamlett, 2002). However, using this method it would be very laborious to make measurements of SST number and size, and to be accurate all sections would need to be cut in exactly the same plane. Consequently, whole mount preparations are preferable when making measurements of this nature (Briskie and Birkhead, 1993). Both techniques yielded similar results when looking for the presence/absence of SSTs in a particular region, suggesting that both are of equal accuracy.

Unfortunately, sperm were never seen within the sperm storage sites. This was not unexpected when using the whole mount technique, as the tissue used was very old and had been stored in ethanol for several decades. Consequently, visibility within the SSTs was probably much reduced (Briskie and Birkhead, 1993). Although past researchers have succeeded in observing sperm within SSTs following histological sectioning of the tissue (e.g. Adams and Cooper, 1988; Conner and Crews, 1980; Cuellar, 1966b; Fox, 1963; Girling et al., 1997; King, 1977; Sever and Hamlett, 2002), success will be largely dependent upon the angle at which the sperm are cut, the degree of differentiation achieved through staining and also a certain degree of luck over whether the contents adhere to the slides during processing. Since the morphology of the structures identified as SSTs in this study was very similar to that seen previously in New Zealand geckos (Girling et al., 1997, 1998; MacAvoy, 1976) and other reptiles (Adams and Cooper, 1988; Conner and Crews, 1980; Cuellar, 1966b; Fox, 1963; Gist and Jones, 1987; Sever and Hamlett, 2002; Shanthakumari et al., 1990), I am confident that these structures have the potential to function as sperm storage areas. SSTs have not previously been recognised in the uterus (Girling, 2002; Sever and Hamlett, 2002), so there is a possibility that tubules located in the uterus function as glands rather than

sperm storage sites (Girling et al., 1997; MacAvoy, 1976; Sever and Hamlett, 2002). However, since sperm need to pass through the uterus to reach the uterine tube SSTs, and invaginations in the uterus did not appear to differ from those located in the vagina and uterine tube, there is no reason to assume that they could not function as SSTs. Consequently, I included these in the analyses.

There was a large amount of intraspecific variation in SST morphology within *Hoplodactylus maculatus*, with the total number of SSTs varying from 14-1901, a more than four-fold variation in SST length and a 13-fold difference in SST area. Intraspecific variation of a similar magnitude has been found in *Anolis carolinensis* (Conner and Crews, 1980; Fox, 1963). This variation cannot be explained by differences in body size, as the only variable significantly related to SVL was the size of SSTs in the uterus, where a negative correlation was observed. There is no obvious explanation for this unexpected negative relationship. However, the fact that such a relationship was not found in the cross-species comparison could suggest that this is not a real effect and is simply due to the relatively small sample size used. Results also suggest that intraspecific variation in SST morphology is not a seasonal effect, as there was no significant difference between months in the number or size of SSTs. In addition, although there was a difference in the number of vaginal folds and the length of SSTs in the uterine tube between the mating and non-mating season, in both cases this was caused by a greater number/size during the non-mating season. This finding is contrary to expectation, as MacAvoy (1976) found that the lumen of sperm storage areas increased in size during the storage season in *H. pacificus* (= *H. maculatus* (Robb and Rowlands, 1977)). Furthermore, where seasonal variation in SSTs has been found in birds, the trend is for an increased size during the breeding season (Birkhead et al., 1997; Briskie, 1994, 1996). This variation may reflect the changing requirements of the oviduct as it prepares for ovulation (Cree A, pers. comm.). Alternatively, other as yet untested factors that might result in such a large degree of variation include the age and physical condition of the individual (Briskie, 1994; Shugart, 1988).

SSTs were found throughout the oviduct in all 16 species studied, suggesting that all are capable of storing sperm at least within a season. In *H. maculatus*, although SSTs were similar in size between regions of the oviduct, they varied greatly in number, with more storage sites located in the uterine tube and uterus than the vagina. A similar

pattern was observed in all the species analysed. Previous studies of New Zealand geckos have shown that the morphology of SSTs is very simple in the vagina, but that tubules in the uterine tube possess glands at their base (Girling et al., 1997, 1998; MacAvoy, 1976), suggesting a more specialised function. It has also been proposed that there is temporal variation in which sperm storage sites are utilised in *H. maculatus*, with sperm being stored in the vagina over winter and then migrating to the uterine tube storage areas in the spring ready to fertilise the eggs (Girling et al., 1997; MacAvoy, 1976). Similar temporal variation in sperm storage site usage also occurs in the yellow-headed blackbird (*Xanthocephalus xanthocephalus*), where vaginal SSTs fill with sperm first, suggesting a gradient of maturation (Briskie, 1996). Thus, in *Hoplodactylus* spp., the majority of which have a dissociate breeding pattern with mating in autumn and ovulation in spring (Gill and Whitaker, 1996; Robinson, 1985; Rowlands, 1981), this variation in number of SSTs between regions may reflect a difference in function. In contrast, *Naultinus* spp., which mate and ovulate in the spring (Gill and Whitaker, 1996; Robinson, 1985; Rowlands, 1981), may predominantly use SSTs in the uterine tube, as they have a shorter sperm storage period.

Across species, the number of SSTs was positively related to SVL, suggesting that larger individuals have greater sperm storage potential. A similar positive relationship has been observed in birds (Birkhead and Møller, 1992; Briskie and Montgomerie, 1993) and is expected, as larger individuals are likely to have larger oviducts and thus a greater overall sperm storage site area. There was a large amount of interspecific variation in SST morphology, with the total number varying from 346-7641, a three-fold difference in length and a nine-fold variation in area. Once again, this variation could not be explained by temporal factors, as neither SST number (corrected for body size) nor size was related to season.

There was a positive relationship between the number and size of SSTs in the vagina. This relationship cannot be explained by allometric effects, as SST number was corrected for body size. In birds, there is often a negative relationship between these two variables (Briskie and Montgomerie, 1993; Briskie et al., 1997). Proposed explanations for this pattern have included space constraints (Briskie et al., 1997) and sperm competition hypotheses: species in which females have fewer SSTs will experience more intense sperm competition; this in turn may result in increased sperm length in

males, necessitating a concurrent increase in SST size (Briskie and Montgomerie, 1993). Thus, this positive correlation observed in New Zealand geckos was unexpected. However, it has been shown that the number of SSTs in birds is positively correlated with the number of sperm in the male's ejaculate (Birkhead and Møller, 1992). Therefore, it is possible that females have evolved larger SSTs where males produce large ejaculates, allowing sperm competition to continue. It is also possible that this could simply be a reflection of the degree to which vaginal sperm storage sites are used: species with a few small vaginal SSTs may preferentially store sperm in the uterine tube or uterus.

Relative testis volume was not related to SST morphology in any region. This was unexpected, as it was predicted that sperm competition would influence both the number and size of SSTs: in birds, species with more intense sperm competition often have fewer SSTs and/or SSTs of greater length (Briskie and Montgomerie, 1993; Briskie et al., 1997), and Morrow and Gage (2000) found a significant relationship between residual testis size and spermathecal volume in moths. However, despite finding a relationship between mating system and SST morphology, Briskie et al. (1997) also found no relationship between SST morphology and testis size. Since there is a strong correlation between testis size and mating system across taxa (see Chapter 2), this finding was unexpected and may suggest that another as yet unmeasured factor is causing this relationship. Across species of New Zealand geckos, there was a negative relationship between the number of folds in the vagina and relative testis volume during the non-sperm storage season. This finding is difficult to interpret as it suggests that when the sperm storage areas are not in use, species in which sperm competition is more intense have a reduction in the number of folds and thus in the potential area for sperm storage sites. This pattern warrants further investigation in the future to determine whether it is a real effect or related to small sample sizes.

Sperm length was negatively related to the number of SSTs in the uterus and the total number of SSTs, meaning that males belonging to species in which females have fewer sperm storage areas have longer sperm. Briskie and Montgomerie (1992) found a similar relationship in birds, where it is thought that a reduction in the number of SSTs increases sperm competition intensity which, in turn, selects for increased sperm length in males, as longer sperm have been shown to swim faster and thus may be competitively superior (Gomendio and Roldan, 1991; but also see Gage et al., 2002).

Surprisingly, there was no relationship between sperm length and SST size. In other taxa, sperm length is often positively associated with storage site length (Briskie and Montgomerie, 1992, 1993; Briskie et al., 1997; Pitnick and Markow, 1994; Pitnick et al., 1999; Presgraves et al., 1999), suggesting that the two evolve in tandem, allowing storage sites to accommodate sperm of increasing length. The lack of such a relationship in New Zealand geckos might suggest that sperm storage sites are sufficiently large to accommodate longer sperm, i.e. sperm storage areas are ahead of sperm length in the evolutionary arms race. However, the fact that over 50% of species analysed had longer sperm than SSTs in all three regions of the oviduct argues against this. It should be remembered that SST length measurements are minimum values, and their accuracy will depend upon the angle at which they were viewed on the slide. Therefore, these values may be underestimates. However, this is unlikely to have greatly affected the results as the same technique was used in other studies where a significant relationship was observed. Since I was unable to see sperm within the SSTs, I could not ascertain whether storage sites were active or inactive. In birds, SSTs increase in size during the mating season whilst they are actively storing sperm (Birkhead et al., 1997; Briskie, 1994, 1996). Consequently, it is possible that many of the SSTs measured in this study were not active, and thus smaller than they would be when functional. However, the absence of seasonal variation in SST size argues against this. Therefore, it seems likely that this lack of a relationship between the size of SSTs and sperm is real and reflects a difference in the way in which sperm are stored within the storage sites: whereas sperm are often layered in other species (Briskie, 1993, 1994, 1996), it is possible that only one layer of sperm is accommodated by the SSTs of several species of New Zealand geckos and that males may be able to block female SSTs with their sperm, preventing access to other males.

Conclusions

All species of New Zealand gecko analysed possess sperm storage sites throughout their oviducts, reflecting a high potential for sperm competition in this group. There is great variation in SST morphology between regions of the oviduct, suggesting some segregation of function. There is also a large amount of intra- and interspecific variation in sperm storage site morphology in New Zealand geckos. This variation is not related to breeding season. There is also no relationship between SST morphology and relative testis size, indicating that the size and number of SSTs does not vary with the intensity of sperm competition. However, there is a negative correlation between sperm length and both the number of uterine and total number of SSTs, suggesting that in species where males have evolved longer sperm, females have had a concurrent reduction in the number of SSTs. It is possible that this pattern is a result of sperm competition. Moreover, the fact that in many species sperm are longer than SSTs suggests that males may be capable of filling and blocking SSTs, preventing access to the sperm of rival males.

Chapter 5: The Social Mating System of *Hoplodactylus maculatus*

Introduction

Knowledge of the mating system of a species is of fundamental importance when asking questions about the evolution of reproductive characteristics, be it behavioural, ecological, morphological or physiological traits that are of primary interest. Despite this, there are still very few lizard species for which the mating system is known in detail. In the past, there has been a tendency for researchers to mainly focus their attention on a very narrow range of genera predominantly belonging to two families, the Iguanidae and Agamidae, both of which are characteristically territorial (e.g. see review by Stamps, 1983). This has led to a general belief that the majority of lizards are likely to have polygynous mating systems (Ruby, 1981; Schoener and Schoener, 1980; Stamps, 1983). However, more recent research encompassing a wider range of taxa has shown that lizard mating systems span the full spectrum, from monogamy (Bull, 1988) through to polygynandry (Olsson et al., 1994a; Olsson and Madsen, 1998, and references therein).

Over the last three decades there has been increased research interest into the reproductive characteristics of New Zealand lizards. Thus there is now a growing body of literature concerning the reproductive cycles of males and females (e.g. Cree, 1994; Cree and Guillette, 1995; Fawcett, 1972; MacAvoy, 1976; McIvor, 1972; Towns, 1975; Wilson and Cree, 2003), reproductive output (Cree, 1994), reproductive morphology of the female tract (Girling et al., 1997, 1998) and the exact timing of mating (Rowlands, 1981) for several species. Unfortunately, however, there is still very little known about the mating behaviour and social mating system of any species. Although there are several reports available concerning the degree of territoriality in certain species (Hitchmough, 1982; Mainwaring, 1979; Porter, 1988), these are largely anecdotal. Moreover, the fact that a few species are thought to be highly territorial has resulted in the majority of private lizard keepers housing a single male with multiple females (Rowlands R, pers. comm.). Consequently, no information concerning the mating system of a species can be obtained from these captive populations. The courtship behaviour of one species, *Hoplodactylus pacificus*, has been described in detail (Rieppel and Schweiz, 1976). However, this account was based on an observation that did not culminate in copulation. Since all species of New Zealand lizard are now fully protected and many

are considered endangered (Daugherty et al., 1994), a detailed understanding of the mating system of these species is of utmost importance, particularly if captive breeding programmes are to become a conservation tool in the future.

H. maculatus, the common gecko, is the most widespread species of gecko in New Zealand. It is found throughout the North and South Islands and on offshore islands, inhabiting forest, scrub and grassland, from sea-level to 1700 m (Gill and Whitaker, 1996). This taxon is believed to be a complex of several species (Hitchmough, 1991, abstract; Hitchmough, 1997), each of which still awaits formal identification and classification. Due to its abundance, this is also the most widely studied species of gecko in New Zealand, and detailed information is available concerning both its general ecology and reproductive biology. *H. maculatus* is a nocturnal gecko with a lifespan of at least 17 years (Anastasiadis and Whitaker, 1987). Like many other gekkonids, it is capable of vocalising, a characteristic believed to be of importance in social communication in this group (Frankenberg and Werner, 1992; Henkel and Schmidt, 1995). Individuals are often found at high densities in a population (McIvor, 1972; pers. obs.), indicating a certain degree of gregariousness, as found in other gekkonids (Bellairs, 1969; Greenberg, 1943; Kearney et al., 2001). Ovulation occurs in the spring (September-October) and young are born in late summer (January-March) (Cree, 1994; MacAvoy, 1976; McIvor, 1972; Whitaker, 1982). Consequently, it was thought that mating must also occur in spring, to coincide with ovulation (Boyd, 1942; McIvor, 1972). However, it is now known that this species mates in autumn (February-May) (MacAvoy, 1976) and that females store sperm over winter in specialised sperm storage regions (Girling et al., 1997, 1998; MacAvoy, 1976). The fact that this species occurs at such high densities and has obligatory sperm storage means that females could mate with several males within a breeding season, storing the sperm from each and thus setting the stage for sperm competition. This, combined with its wide distribution, renders *H. maculatus* a particularly interesting and suitable study species for the investigation of mating strategies.

In this chapter I investigate the social mating system and reproductive behaviour of *H. maculatus*. The first part of this study focuses on information obtained from a wild population. I have determined the sex ratio, density and associations between individuals of both sexes. The combination of these features has enabled me to make predictions about the likely mating system of this species: if monogamous, I would

expect to observe an even sex ratio, low population density and the formation of single male-female pairs during the breeding season; if polygynous, the population is likely to be female-biased and single males should be associated with several females; if polyandrous, the sex ratio is likely to be male-biased and a single female associated with several males; and if polygynandrous, the sex ratio is likely to be 1:1, the population density high and groups consisting of multiple males and females are likely to be seen.

Secondly, I have obtained data from experimental captive populations. This has provided more detailed information on courtship and mating in this species, including peak activity periods, the degree of territoriality and a description of courtship and mating behaviour. I have investigated whether body size affects male and female reproductive success: in several other lizard species it has been shown that body size can be important in the formation of dominance hierarchies (Andrews, 1985; Brattstrom, 1974; Fox et al., 1981; Manzur and Fuentes, 1979), male and female choice and mating success (e.g. Cooper and Vitt, 1993; Gullberg et al., 1997; Olsson, 1993; Salvador and Veiga, 2001). Finally, I have determined the level of male and female promiscuity in these captive populations and related these findings back to predictions made from the field study.

Methods

Field Survey

A field population located at Devil's Knob, near Birdlings Flat, Banks Peninsula (Fig. 5.1A) was selected for surveys of population parameters in *Hoplodactylus maculatus* (*H. maculatus* "Canterbury") (Fig. 5.2). Devil's Knob is a discrete rock outcrop approximately 45 m long, 18 m wide and 60 m high (Fig. 5.1B). The nearest neighbouring rock outcrop is approximately 60 m away, a distance greater than that travelled by most individuals of this species complex (McIvor, 1972; Whitaker, 1982). Consequently, I was relatively confident that there would be little migration to and from this site between visits. Geckos were found inhabiting vertical and horizontal crevices in regions where the predominant rock type was basalt.

This population was sampled from October 2000 to March 2002. I visited the site at least once a fortnight during the mating season (February-May) and approximately once a month at other times of the year. A circuit of the rock outcrop was made on each visit and all accessible cracks were inspected for geckos. Each circuit took 1.5-4 h to

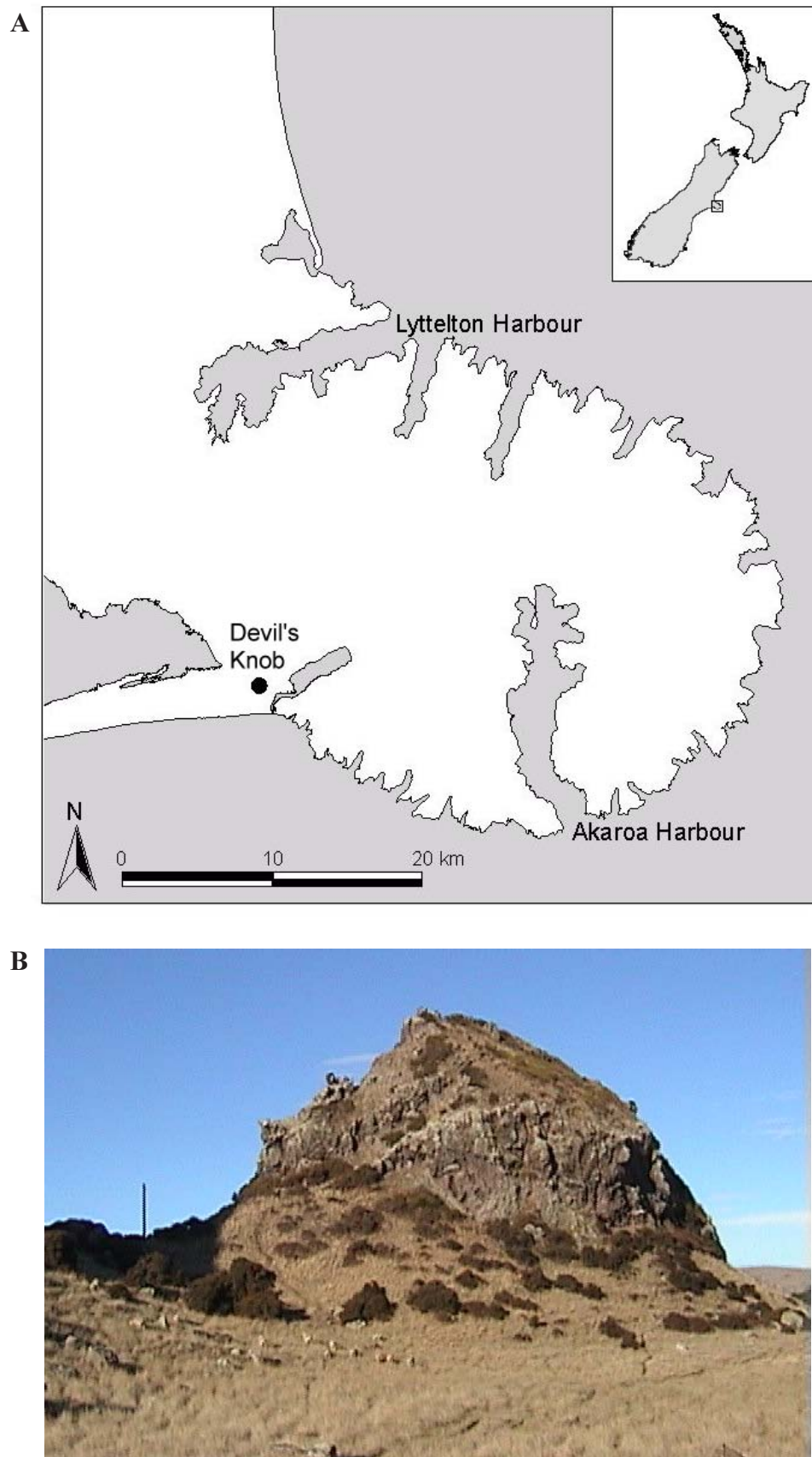


Fig. 5.1: Location map (A) and photograph (B) of the study site at Devil's Knob, near Birdlings Flat, Banks Peninsula.



Fig. 5.2: *Hoplodactylus maculatus*

complete. Through this method I covered approximately 18% of the outcrop. Ambient temperature in the shade was recorded on each visit. Upon sighting an individual, I attempted to extract it from the rock crack using a piece of plastic-coated electrical wire. Upon capture, individuals were placed in a muslin bag. They were then weighed using a 30 g Avinet spring balance and snout-vent length (SVL) and tail length were measured. All individuals greater than 55 mm SVL were classed as adults, as individuals of this size could be readily sexed and this has previously been found to be the minimum size of sexual maturity for another population of *H. maculatus* (Whitaker, 1982). I recorded the sex of each individual: adult geckos can easily be sexed externally, as males have a swollen area at the base of the tail containing the inverted hemipenes, deep orange pigmented preanal glands and two spurs slightly anterior to the cloaca (MacAvoy, 1976; McIvor, 1972; Robb, 1974). Tail condition (original or regenerated) and the presence of ectoparasites were noted.

In order to sample the population accurately, I needed to give each individual a unique identification mark. The traditional method used among herpetologists is toe-clipping. However, since geckos use their toes for climbing, it is possible that this could have a detrimental effect on their foraging success, mate acquisition and survival. The exact impact of toe-clipping on gecko populations is still unknown: Bustard (1971) experienced low recapture success following toe-clipping in the gecko *Oedura ocellata*, possibly indicating that individuals had been lost from the population, whereas no detrimental effects were observed for *Hemidactylus turcicus* (Paulissen and Meyer, 2000). Consequently, it is preferable to avoid this technique wherever possible.

Therefore, I used temporary marks. Individuals were initially painted with a unique combination of numbers and dots using ‘Twink’ (white correction fluid). However, upon drying this tended to crack and flake off, so that marks were only retained for a few weeks. Therefore, for the majority of the study white enamel model paint (‘Humbrol Super Enamel’) was used. In some lizards the use of enamel paint has been linked to necrosis (Olsson M, pers. comm.); however, there was no evidence of any detrimental effects in this study. Paint marks were retained until moulting, at which time they were shed with the old skin. During the summer, moulting occurs approximately once every 4-8 weeks (Mainwaring, 1979; Robb, 1974; Rowlands, 1981; Whitaker, 1976, 1982; pers. obs.) and individuals tend to moult at least 3-4 times a year (pers. obs.). Therefore, I was unable to follow the same individual across years. However, since this study was of a relatively short duration, I was able to reliably estimate recaptures through size, sex and tail condition information.

Results from the field survey were used to estimate population parameters including population structure, sex ratio and density. In addition, estimates of site fidelity and intersexual interactions were made. Wherever month was included in combination with other factors, data was analysed using split-plot ANOVAs, using day as the replicate of month. F-tests were used in all cases as, although the majority of data was count data, it was normally distributed. Where there were only two possible outcomes, binomial tests were used.

Enclosure Study

In total, five captive populations of *H. maculatus* were maintained during 2001 and 2002. Individuals were collected from rock outcrops around Banks Peninsula. Only individuals larger than 60 mm SVL were collected. Although it would also have been preferable for only individuals with original tails to have been used, as tail loss has been shown to affect reproductive success in lizards (Martín and Salvador, 1993; Salvador et al., 1996), this was not feasible due to the difficulties encountered in locating a sufficient number of individuals within a short time-frame. However, only individuals with well regenerated tails ($\geq 72\%$ of SVL) were collected. Geckos were transported from the field to enclosures in a muslin draw-string bag. Each individual was painted with a large number on its back to enable rapid identification from a distance.

During 2001, three populations of geckos were housed in outdoor, purpose-built enclosures (Fig. 5.3A). Enclosure design followed recommendations from the Department of Conservation (Anonymous, 1999) and was similar to that used by Porter (1988). Enclosures were constructed from untreated timber. Three walls and the roof (in two parts for access) were covered with an inner layer of 1 mm insect screen and an outer layer of 13 mm predator-proof chicken mesh; the fourth wall was a Perspex viewing screen. The floor was made from 70% shade cloth. A false vertical plywood wall was placed within each enclosure and 12 shelter boxes (1 L ice-cream containers) were attached to the back of this. Each box had a transparent Mylar viewing section cut into its lid. The back of each enclosure was covered with black polythene during the day; this was converted into a tent-like construction during the night to provide shelter from external light sources whilst still allowing viewing access to each box. Shade cloth was used to shelter 50% of the enclosure from the sun during the day. Enclosures were furnished with pot plants (*Coprosma propinqua* and *Muehlenbeckia astonii*) and branches, forming perching sites.

Each enclosure contained five males and five females at a density of approximately 4 individuals/m³ or 1.18/m² (floor area plus four walls). These were monitored using a Sony camcorder with an infrared light source. The size of these enclosures meant that they could only be monitored in person. Although many interactions were noted, only one mating event was seen during this first season. Consequently, modifications were made to the enclosure design the following season.

During 2002, two smaller enclosures were constructed within two of the original structures (Fig. 5.3B). Two of the original insect screen / chicken mesh walls were used; the remaining two walls were made of Perspex. A 15 cm rim of galvanised sheet metal at an angle of 35° ran around the top of the walls, to prevent geckos from escaping. Plywood shelters were placed on the floor. This reduction in enclosure size meant that permanent video cameras could be installed, eliminating the possibility that the paucity of mating observations during the first season was due to disturbances caused by my presence. Each enclosure had two cameras. The first had a wide-angle lens attached and monitored the whole enclosure from above. This was linked to a time-lapse VCR, enabling a 12 h period to be recorded onto a 3 h tape. The second viewed the inside of a focal shelter through holes cut into the Perspex walls; this camera was linked to a standard VCR. Each video camera had an inbuilt infrared light. However, an additional

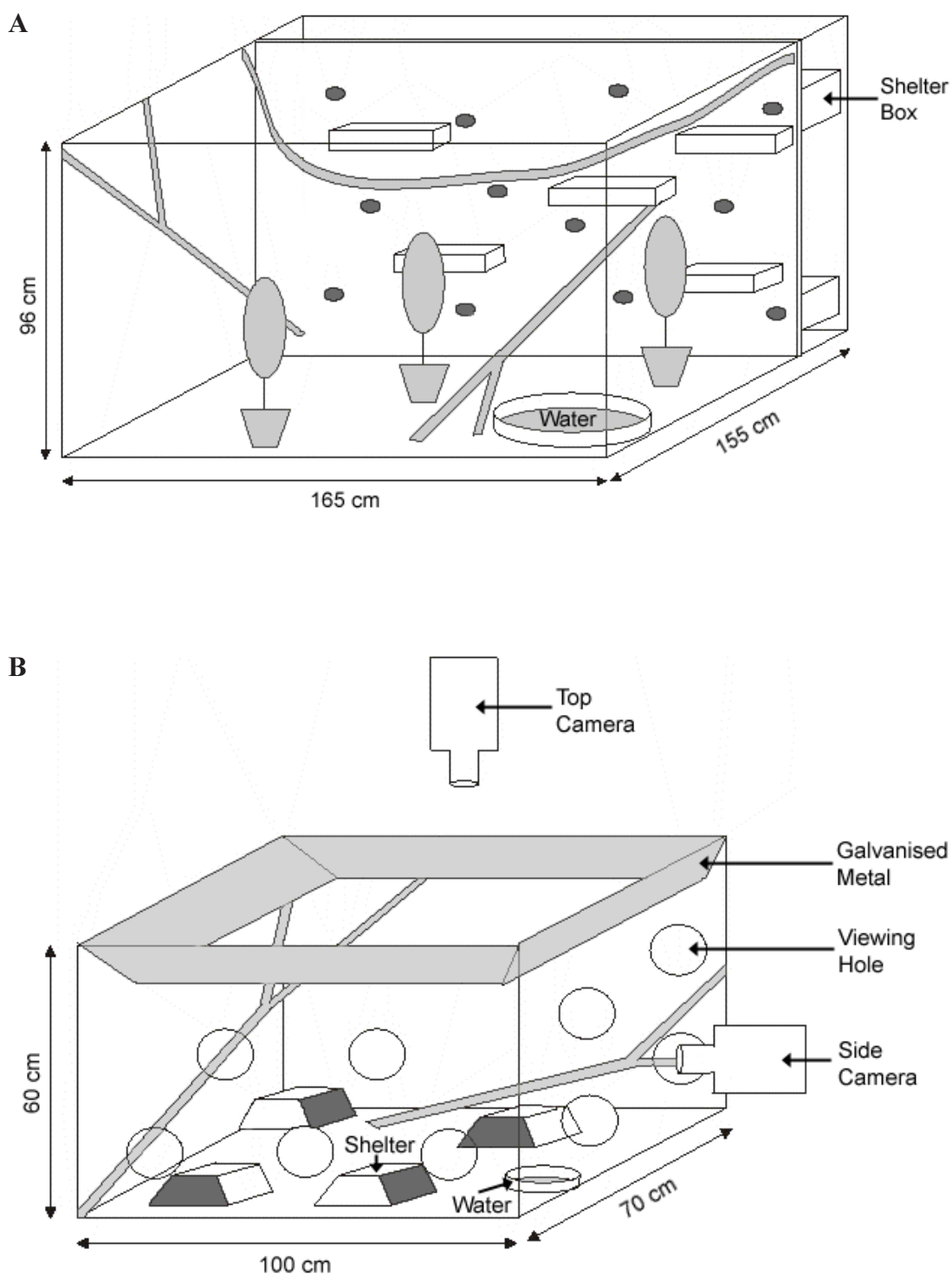


Fig. 5.3: Diagram of enclosure design (not to scale) during: A. 2001; and B. 2002.

infrared light source was required to facilitate night vision. The enclosures still housed five individuals of each sex, resulting in an increased density of approximately 24 individuals/m³ or 3.65/m².

Geckos were predominantly maintained on a diet of moths and honey. However, during the winter, when moths were largely unavailable, they were fed house flies. Water was available at all times both in permanent water bowls and through misting of the enclosures. 'Bone-gro' was occasionally added to honey to ensure that females were obtaining sufficient calcium and 'Ornithon' vitamin supplement was periodically added to the water prior to misting. All young born in captivity were immediately removed from the enclosures, to avoid the risk of predation by other geckos, and placed indoors in glass aquaria. These were maintained on wingless *Drosophila*.

During the first season, enclosures were monitored from October 2000 to October 2001. This allowed me to determine the exact timing of mating for this species. I initially concentrated my observations around the hours of darkness, as this species is believed to be fully nocturnal (McIvor, 1972, 1973). However, I found that a large amount of activity also occurred during the day, both whilst basking and also within the shelter boxes. Consequently, from February 2001 enclosures were monitored during both the day and night. Through these observations I found that the majority of behavioural interactions occurred between February and April. Therefore, during 2002 enclosures were monitored during these months. In addition, behavioural observations were made during September and October to ensure that there was no additional spring mating period.

During each observation period, notes were made of all interactions between individuals and the location of individuals at the end of the activity period, allowing me to determine the degree of territoriality. Within and between years, each enclosure was observed for different lengths of time both in total and between time periods. Consequently, all results are expressed as rates, corrected for the number of hours of observation. As there was a relatively high degree of variability between years in terms of climatic conditions, population density and methodology, year is included as a factor in all analyses, using a split-plot design with enclosure as the source of replication. To assess temporal variation in climatic conditions, maximum and minimum ambient temperatures at the enclosure site were recorded approximately daily during the months of observation.

Although two cameras were used during 2002, results presented are those collected from the top camera, as the side camera only focussed on one shelter at a time and thus may have introduced bias to the results, e.g. the focal shelter may have been frequented more by some individuals than others or may have been more/less frequented than other shelters. Since only a small proportion of all interactions were observed by the side camera (mean = 18.4%), this is unlikely to have affected the results. Unfortunately, during 2001 one male died in March. Consequently, this enclosure was removed from all analyses to prevent it biasing the results in any way.

Results

Field Survey

In total, I captured 139 individuals during the course of this study. Overall, the population was female-biased, with a sex ratio of approximately 2:1. There was slight variation in the average number of individuals captured between months (range = 0-9 individuals) (Fig. 5.4). However, this difference was not significant (split-plot ANOVA using day as replicate for month: $F_{14,22} = 1.53$, $P = 0.18$). There was a significant difference between the sexes in the number of individuals caught ($F_{2,44} = 9.74$, $P < 0.001$), with significantly more females being caught than males (Tukey Test: $q_{44,3} = 6.24$, $P < 0.001$; mean daily capture \pm SE: female: 2.51 ± 0.31 ; male: 1.13 ± 0.20 ; immature (unknown sex): 1.84 ± 0.20). There was no interaction between month and sex ($F_{28,44} = 0.70$, $P = 0.84$), meaning that this sex ratio bias was consistent across months. Similarly, there was no significant

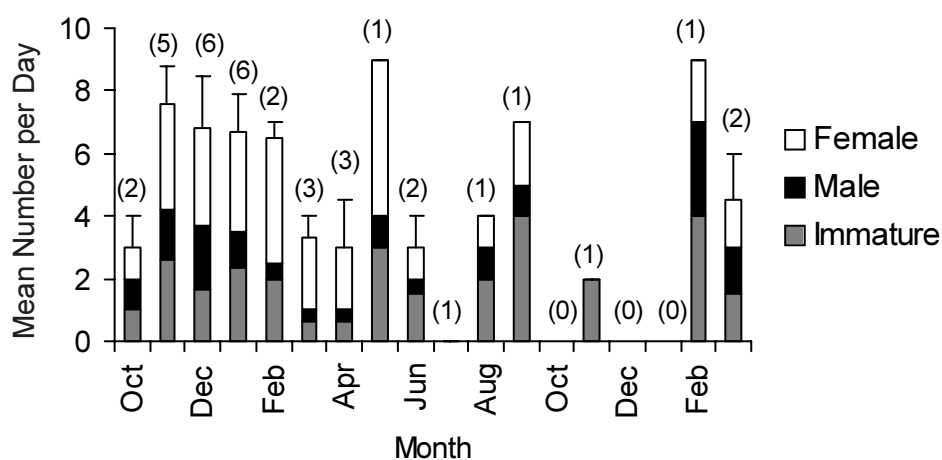


Fig. 5.4: Mean (\pm SE) number of individuals of *Hoplodactylus maculatus* captured across months during 2000-2002 at the field site. Standard errors represent variation in the total number of individuals captured. Number of days sampled in parentheses.

difference in capture success between months in terms of the proportion of those individuals sighted that were successfully captured (binomial response - number captured of total number sighted: $\chi^2_{14} = 14.29$, $P = 0.43$).

It appeared that capture success was influenced by environmental conditions. However, there was no significant relationship between the number of individuals caught and temperature ($F_{1,93} = 1.83$, $P = 0.18$). There was still a significant difference between the sexes ($F_{2,93} = 9.83$, $P < 0.001$), but no interaction between temperature and sex ($F_{2,93} = 0.84$, $P = 0.44$), meaning that this sex bias was not temperature dependent. Similarly, temperature did not affect the proportion of those individuals sighted that were successfully captured (binomial: $\chi^2_1 = 0.009$, $P = 0.93$).

The ratio of juveniles:adults captured also varied between months (mean monthly range = 0.63:1 to 2:1). However, this difference was not significant ($\chi^2_{13} = 11.07$, $P = 0.61$).

The overall density of individuals on the outcrop was 0.47 individuals/m². However, there was great variation in density between regions (range = 0-5.56/m²). During the course of the study, multiple males and females were found to inhabit the same region of outcrop, the maximum number being six males and 15 females in a 7.5 m² area. Within the same crack on the same day, the following sex combinations were observed: two females ($n = 4$); one male and one female ($n = 6$); two females and one male ($n = 1$); and two males and one female ($n = 1$).

In total, 22 adults and nine juveniles were recaptured with their original marks. Some individuals were caught up to six times, and there was as much as nine months between captures in some cases. From these recaptures, I found that individuals tended to move very little. Of the adults, 13 were located in the same crack as their initial capture site and five had moved less than 1 m. None of the juveniles had changed location.

There was no significant difference in the size of males and females in the population (mean SVL \pm SE - male: 57.79 ± 0.96 mm; female: 57.64 ± 0.70 mm; $t_{64} = -0.13$, $P = 0.90$) (Fig. 5.5). Overall, 52% of individuals in the population had ectoparasites. There was no significant difference in the number of individuals infested either between the sexes ($\chi^2_1 = 0.39$, $P = 0.53$) or between adults and juveniles ($\chi^2_1 = 0.24$, $P = 0.62$). Tail loss was common in this population, with 62% of individuals having a regenerated tail. There was no difference between the sexes in the incidence of tail loss ($\chi^2_1 = 0.32$,

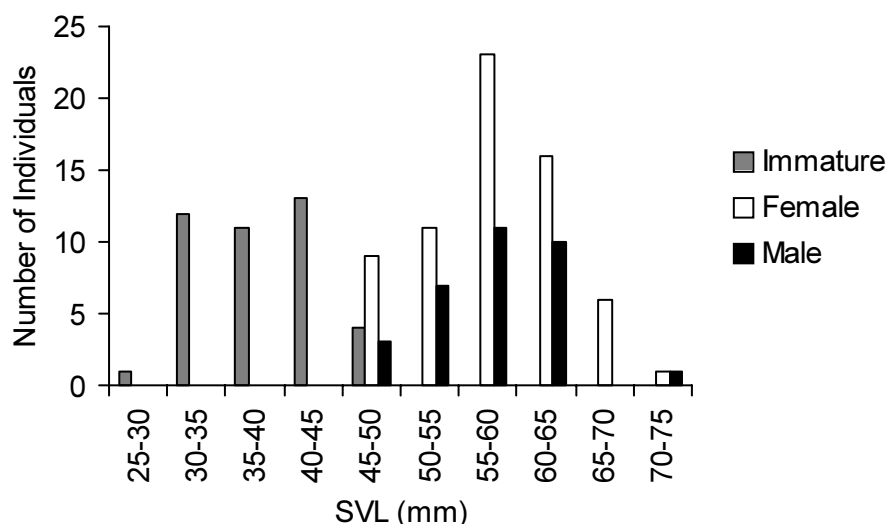


Fig. 5.5: Size distribution of *Hoplodactylus maculatus* in the field population at Devil's Knob.

$P = 0.57$). However, there was a significantly greater proportion of regenerated tails amongst adults than juveniles ($\chi^2_1 = 7.17$, $P = 0.007$).

One mating event was observed in the field. This occurred on 8th April 2001 at 11.02 am. The pair was seen mating on the rock face, close to a crevice.

Enclosure Study

a) 2001/2002 temperature

Since this study spanned two seasons, it is important to determine whether any extrinsic factors may have affected the distribution of observed behaviours. One obvious environmental condition expected to affect lizard behaviour is temperature. A comparison of maximum and minimum temperature (Fig. 5.6) shows that overall it was slightly warmer in 2002 from February-May. Maximum temperature did not differ significantly between years ($F_{1,123} = 2.49$, $P = 0.12$). There was, however, a significant difference between months ($F_{3,123} = 11.77$, $P < 0.001$) and a significant interaction between month and year ($F_{3,123} = 4.31$, $P = 0.006$), the warmest month being February in 2001 and March in 2002. There was a significant difference between years in minimum temperature ($F_{1,123} = 10.68$, $P = 0.001$), with 2001 being cooler than 2002 (Mean \pm SE - 2001: $9.89 \pm 0.53^\circ\text{C}$; 2002: $11.38 \pm 0.30^\circ\text{C}$). There was also a significant difference between months. Overall, March had the highest minimum temperature ($12.67 \pm 0.33^\circ\text{C}$) and May the lowest ($8.21 \pm 0.69^\circ\text{C}$). Both February and March had significantly higher minima than April and May (Tukey Test - February x April: $q_{123,4} = 6.63$, $P < 0.001$; February x May: $q_{123,4} = 9.47$, $P < 0.001$; March x April: $q_{123,4} = 7.49$, $P < 0.001$;

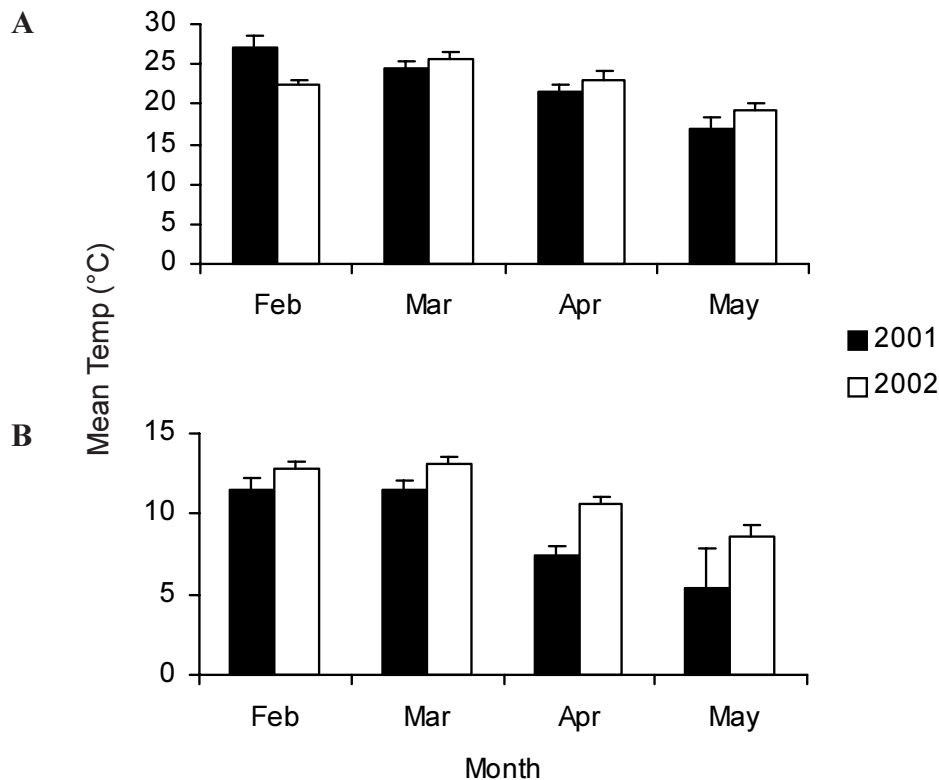


Fig. 5.6: Mean (\pm SE) temperature during 2001 and 2002: A. Maximum; B. Minimum.

March x May: $q_{123,4} = 10.23$, $P < 0.001$). There was no significant interaction between month and year ($F_{3,123} = 1.37$, $P = 0.25$).

b) Courtship behaviour

During the course of this study, a large number of interactions were observed between individuals. Many of these were stereotypical behaviours that have been identified in other lizards as courtship behaviour. For the purpose of this study, I identified eight behaviours that typically occurred between males and females and fitted other descriptions of courtship behaviour, both in this (Rieppel and Schweiz, 1976) and other (Carpenter and Ferguson, 1977; Cogger, 1978; Greenberg, 1943; Mayhew, 1966) species:

1. Male approaches female jerkily or hesitantly;
2. Female approaches male jerkily or hesitantly;
3. Male trembles/shakes his head or body at the female;
4. Female trembles/shakes her head or body at the male;
5. Male sits on female's back and holds or nibbles her;
6. Male sits on female's back and bites her firmly in the flank or back of the neck;
7. One individual licks the other's cloaca;
8. Male on female's back with tails entwined / mating.

During the two seasons, only one definite mating was observed in the enclosures. This occurred on 7th February 2001, at 2.49 am. Since the pair were first seen whilst mating, no description of events prior to this is possible. The male was sitting on the female's back with his tail entwined around hers. They separated 3 min after first being observed. The female then moved onto the male's back and both swished their tails from side to side. The male then moved away. Approximately 2 min later, the male approached the female again and she kicked him with her back foot. He bit her on the back and twitched his head; she bit him back and they separated.

During 2001, three other possible matings occurred, where the male had his tail entwined around the female but intromission could not be determined. These occurred on 6th February (11.50 pm), 6th April (7.50 am) and 20th April (7.51 am). During 2002, two additional possible matings were observed: 21st February (1.52 pm) and 13th March (1.07 am). Thus it appears that matings occur from February-April, and may occur throughout the 24 h period.

Seasonal pattern of courtship behaviour:

During the first season, enclosures were monitored from October 2000 to October 2001, enabling me to determine peak activity periods. There was a significant difference between months in the number of courtships per hour of observation (randomised block ANOVA (enclosure = block): $F_{12,12} = 4.33$, $P = 0.008$) (Fig. 5.7). No courtship activity was observed from October 2000-January 2001 or during June 2001, and peak courtship activity was seen during February.

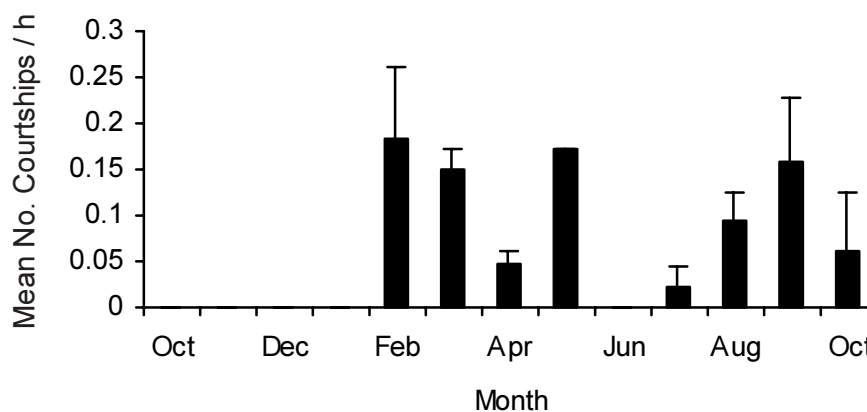


Fig. 5.7: Mean (\pm SE) number of courtships/h across months from October 2000-2001 in captive populations of *Hoplodactylus maculatus*.

Temporal variation in courtship behaviour:

Enclosures were observed from February-May and September-October during both seasons. By breaking the number of courtships per hour down into year, month and time block (midnight-6 am; 6 am-12 noon; 12 noon-6 pm; and 6 pm-midnight), I was able to assess temporal variation in courtship activity. Since the true source of replication for each year was the enclosures, I used a split-plot experimental design in my analysis, with a square-root transformation to normalise the data. There was a significant difference between years ($F_{1,2} = 46.58$, $P = 0.02$), with higher levels of courtship activity in 2001 than 2002 (2001: $0.13 \pm 0.04/\text{h}$; 2002: $0.02 \pm 0.004/\text{h}$). There was no significant difference between months ($F_{5,46} = 1.57$, $P = 0.19$) or time blocks ($F_{3,46} = 0.57$, $P = 0.64$) and none of the interactions were significant (Year x Month: $F_{5,46} = 0.62$, $P = 0.69$; Year x Time: $F_{3,46} = 1.73$, $P = 0.17$; Month x Time: $F_{15,46} = 0.93$, $P = 0.54$; Year x Month x Time: $F_{15,46} = 0.86$, $P = 0.61$).

Male body size and courtships:

There are two possible measures of body length in lizards: SVL and total length. In the following analyses, lizards of the same sex within each enclosure were ranked from 1-5 on the basis of each of these measures, rank 1 being the smallest and rank 5 the largest individual.

(i) SVL ranks:

Amongst males, there was a significant difference between years ($F_{1,2} = 18.92$, $P = 0.048$) and size ranks ($F_{4,8} = 8.86$, $P = 0.005$) in the number of courtships/h. The interaction between year and size was also significant ($F_{4,8} = 10.28$, $P = 0.003$): during 2001 males belonging to the largest size category displayed more courtship behaviour, whereas there was little difference between categories in 2002 (Fig. 5.8).

(ii) Total body length ranks:

The mean number of courtships per hour varied with both year ($F_{1,2} = 18.92$, $P = 0.049$) and size rank ($F_{4,8} = 16.26$, $P < 0.001$). There was a significant interaction between year and size ($F_{4,8} = 11.05$, $P = 0.002$). The largest size class of male had the greatest rate of courtship behaviour during both years; the second largest class had the lowest rate in 2001, but a relatively high rate in 2002 (Fig. 5.9).

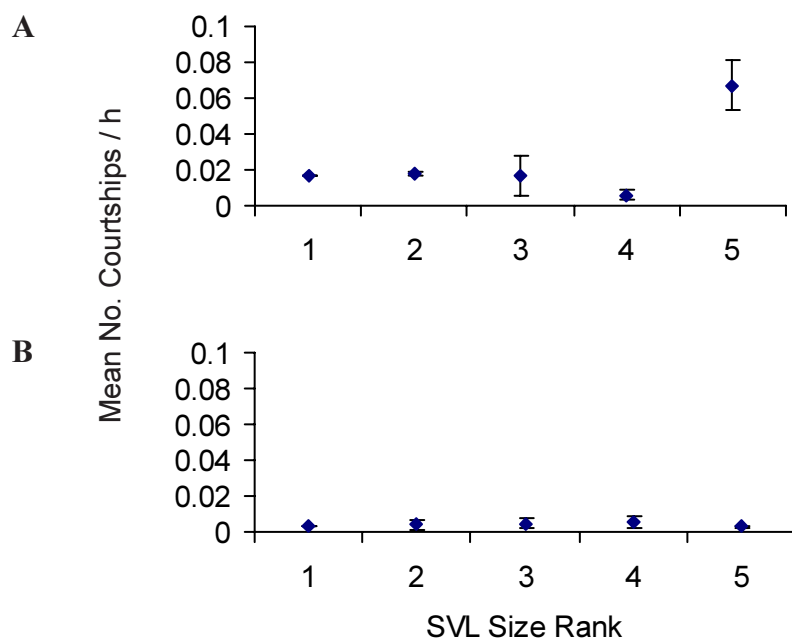


Fig. 5.8: Relationship between size rank (SVL) and courtship rate in male *Hoplodactylus maculatus* during: A. 2001; and B. 2002. Size rank 5 is larger than rank 1.

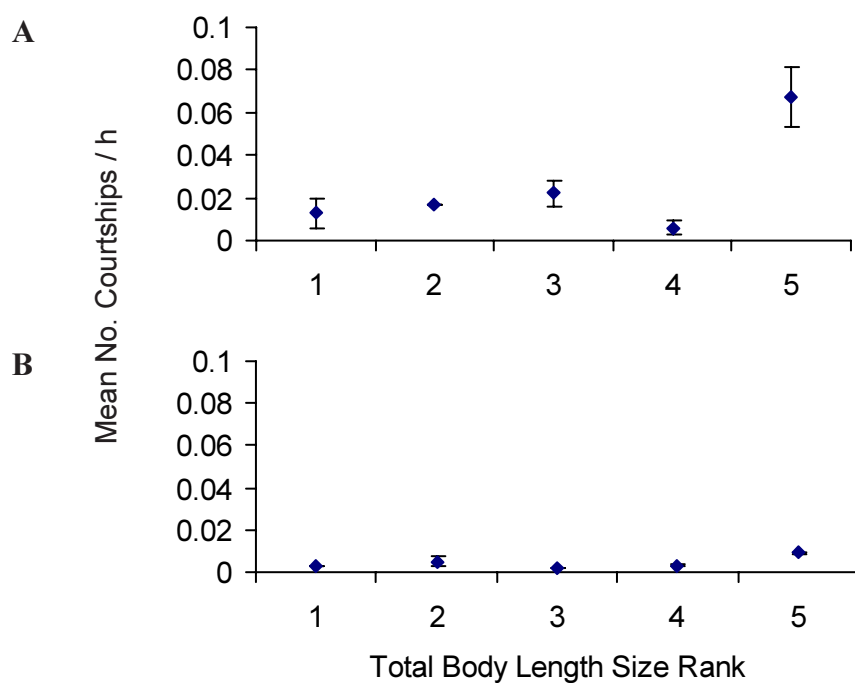


Fig. 5.9: Relationship between size rank (total body length) and mean (\pm SE) courtship rate in male *Hoplodactylus maculatus* during: A. 2001; and B. 2002. Size rank 5 is larger than rank 1.

Female body size and courtships:

(i) SVL ranks:

There was a significant difference between years in the courtship rate of females ($F_{1,2} = 22.97$, $P = 0.04$), with a greater rate in 2001 than 2002 (2001: $0.02 \pm 0.004/h$; 2002: $0.004 \pm 0.0008/h$). There was no difference in courtship rate between females of different size ranks ($F_{4,8} = 2.57$, $P = 0.12$) and no interaction between year and size ($F_{4,8} = 3.60$, $P = 0.06$).

(ii) Total body length ranks:

As above, there was a significant difference between years in the courtship rate of females. There was also a significant difference in courtship rate between females belonging to different size classes ($F_{4,8} = 10.48$, $P = 0.003$) and a significant interaction between year and size ($F_{4,8} = 5.07$, $P = 0.02$). During both years the middle size class females had the greatest courtship rate. The lowest rate was seen in the second smallest size class during 2001 but the largest size class during 2002 (Fig. 5.10).

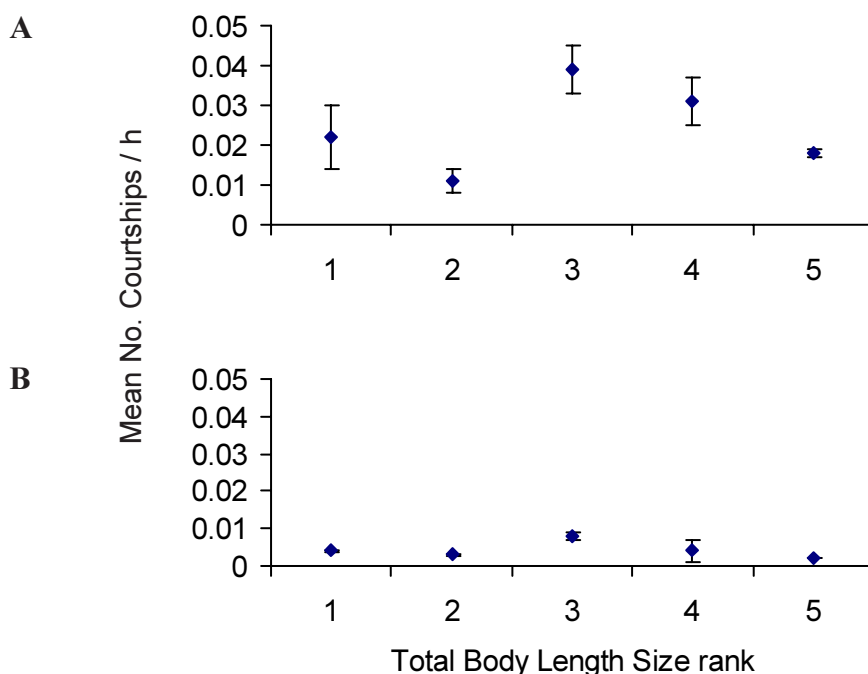


Fig. 5.10: Relationship between size rank (total body length) and mean (\pm SE) courtship rate in female *Hoplodactylus maculatus* during: A. 2001; and B. 2002. Size rank 5 is larger than rank 1.

Number of partners:

Within each enclosure, each individual had the opportunity to court or be courted by up to five individuals of the opposite sex. Consequently, in the following analyses a binomial response was used (number courted / total possible (=5)). Where data did not meet the assumptions of the binomial test, an F-test approximation was calculated.

On average, each male courted and each female was courted by 3.05 ± 0.17 members of the opposite sex. The number courted/courting did not vary significantly between years (No. females courted: $F_{1,2} = 5.08$, $P = 0.15$; No. males courting: $\chi^2_1 = 1.05$, $P = 0.30$) or enclosures (No. females courted: $F_{2,16} = 0.21$, $P = 0.81$; No. males courting: ($\chi^2_2 = 0.41$, $P = 0.81$).

c) Fight behaviour

In gekkonids, many of the behavioural elements associated with fighting are also seen during typical courtship interactions, such as head twitching and biting (Carpenter and Ferguson, 1977; pers. obs.). Consequently, only interactions involving chasing, biting or wrestling between members of the same sex were classified as fight behaviour.

Seasonal pattern of fight behaviour:

During 2001, there was no significant difference between months in the number of fights observed per hour of observation (randomised block ANOVA: $F_{12,12} = 1.47$, $P = 0.26$).

Temporal variation in fight behaviour:

There was no significant difference in fight rate (sqrt transformed data) between years ($F_{1,2} = 0.04$, $P = 0.86$). There was, however, a significant difference between time periods ($F_{3,46} = 2.90$, $P = 0.04$) and a significant interaction between year and time ($F_{3,46} = 7.10$, $P < 0.001$), the 6 pm-midnight time period displaying the lowest fight rate during 2001 and the greatest rate during 2002 (Fig. 5.11). In addition, there was a significant difference between months ($F_{5,46} = 5.67$, $P < 0.001$) and a significant interaction between month and time of day ($F_{15,46} = 2.09$, $P = 0.03$), the 6am-12 noon time period having the greatest fight rate during February-April and September and the

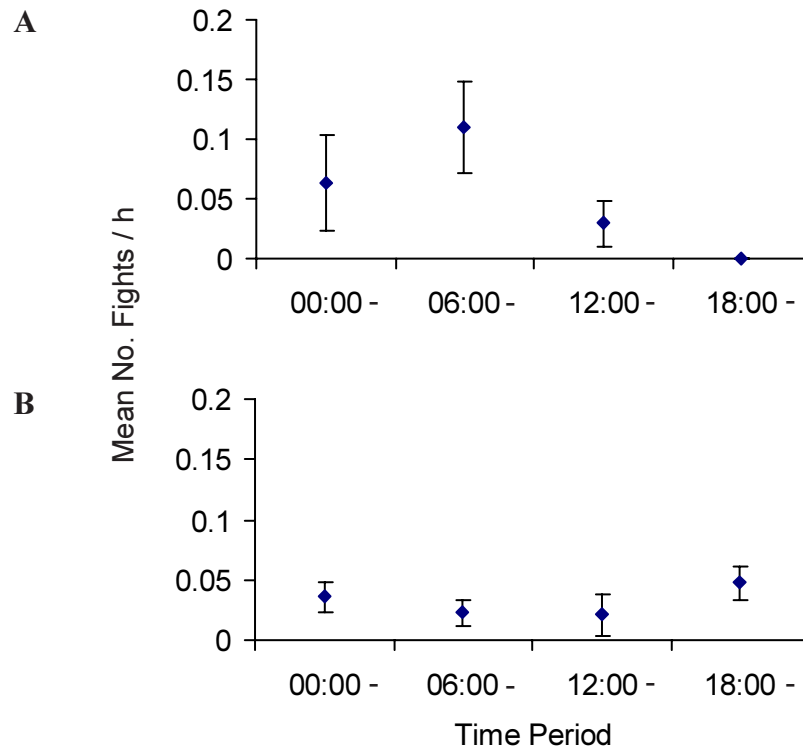


Fig. 5.11: Temporal variation in fight rate of *Hoplodactylus maculatus* during: A. 2001; and B. 2002.

lowest rate during October (Fig. 5.12). No other interaction was significant (Year x Month: $F_{5,46} = 0.20$, $P = 0.96$; Year x Month x Time: $F_{15,46} = 1.56$, $P = 0.12$).

Intersexual variation in fight behaviour:

There was no significant difference in fight rate between years (Year: $F_{1,2} = 5.68$, $P = 0.14$), and males and females fought at a similar rate ($F_{1,2} = 0.64$, $P = 0.51$). There was no interaction between year and sex ($F_{1,2} = 1.46$, $P = 0.35$).

Body size and attack behaviour:

To determine the effect of body size on the likelihood of an individual attacking another, the relative sizes of attackers and individuals being attacked were compared. Two methods were used. Firstly, the relative size difference was used, i.e. whether the attacker was larger or smaller than the individual it was attacking; secondly, the difference in ranks was calculated, so that the magnitude of difference could be compared (where a positive difference indicates that the attacker is larger than the individual being attacked). Once again, two measures of body size were used: SVL and total body length. Year, sex and their interactions were included in all analyses.

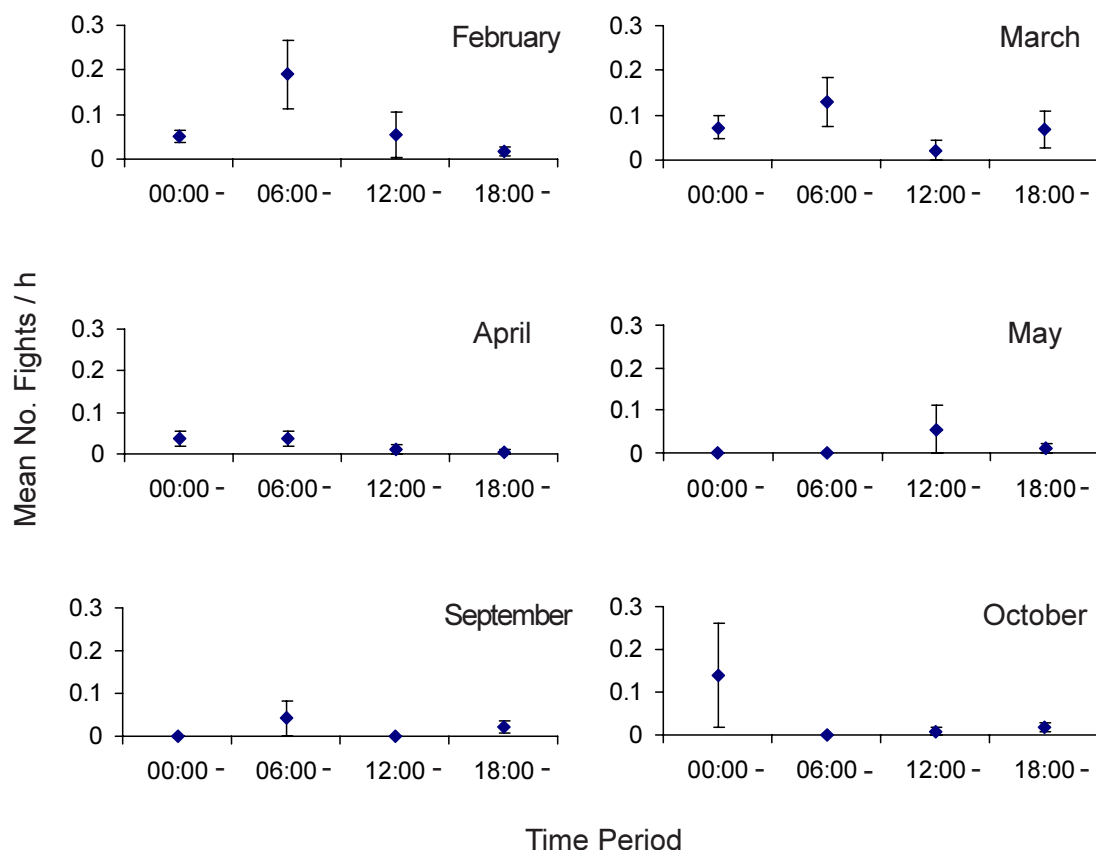


Fig. 5.12: Relationship between time of day and mean (\pm SE) fight rate in *Hoplodactylus maculatus* across months.

(i) SVL rank:

The number of attacks did not vary significantly between years ($F_{1,2} = 0.26$, $P = 0.66$) or the two sexes ($F_{1,6} = 0.58$, $P = 0.48$) and there was no significant interaction between year and sex ($F_{1,6} = 0.90$, $P = 0.38$). The relative size of an individual did not affect the likelihood that they would attack another, i.e. an individual was equally likely to attack another larger or smaller than itself ($F_{1,6} = 1.00$, $P = 0.36$). There was no significant interaction between relative size and any other variable (Year x Size: $F_{1,6} = 0.10$, $P = 0.76$; Sex x Size: $F_{1,6} = 0.08$, $P = 0.79$; Year x Sex x Size: $F_{1,6} = 0.22$, $P = 0.66$).

When the rank difference between attacker and attacked was used in the analysis, once again there was no significant difference between years ($F_{1,2} = 0.26$, $P = 0.66$), the sexes ($F_{1,30} = 1.20$, $P = 0.28$) or their interaction ($F_{1,30} = 1.87$, $P = 0.18$). The likelihood of an individual attacking another was not dependent on the size rank difference ($F_{7,30} = 1.72$, $P = 0.14$), and there was no significant interaction between size and any other factor (Year x Size: $F_{7,30} = 0.69$, $P = 0.68$; Sex x Size: $F_{7,30} = 0.37$, $P = 0.91$; Year x Sex x Size: $F_{7,30} = 0.47$, $P = 0.85$).

(ii) Total body length rank:

There was no significant difference between years ($F_{1,2} = 0.35$, $P = 0.61$) or the sexes ($F_{1,6} = 0.86$, $P = 0.39$) in attack rate, and no interaction between year and sex ($F_{1,6} = 2.10$, $P = 0.20$). However, the relative size of an individual did significantly affect attack rate ($F_{1,6} = 9.95$, $P = 0.02$), with individuals being more likely to attack another smaller than themselves (mean attack rate - larger: $0.01 \pm 0.002/\text{h}$; smaller: $0.004 \pm 0.001/\text{h}$). There was no significant interaction between relative size and any other factor (Year x Size: $F_{1,6} = 1.05$, $P = 0.35$; Sex x Size: $F_{1,6} = 0.23$, $P = 0.65$; Year x Sex x Size: $F_{1,6} = 0.001$, $P = 0.97$).

When the difference in ranks of the attacker and the individual being attacked was compared, once again there was no significant difference between years ($F_{1,2} = 0.26$, $P = 0.66$) or the sexes ($F_{1,30} = 1.54$, $P = 0.22$) in the attack rate, and no significant interaction between year and sex ($F_{1,30} = 2.39$, $P = 0.13$). However, rank difference had a significant effect on attack rate ($F_{7,30} = 3.28$, $P = 0.01$), with differences of -1 to +4 having the greatest attack rate (Fig. 5.13). There was no interaction between size and any other factor (Year x Size: $F_{7,30} = 1.55$, $P = 0.19$; Sex x Size: $F_{7,30} = 0.70$, $P = 0.37$; Year x Sex x Size: $F_{7,30} = 0.81$, $P = 0.59$).

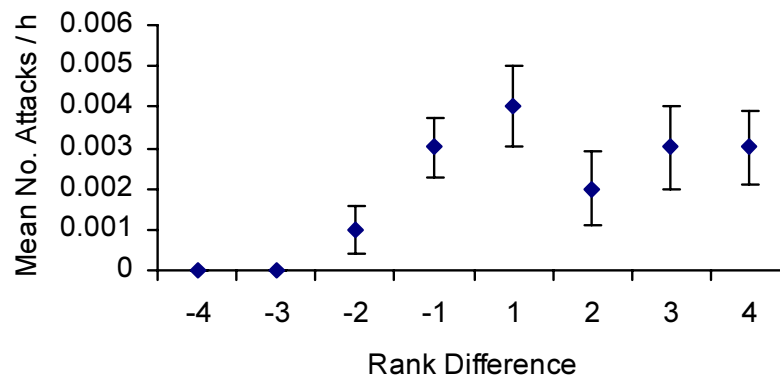


Fig. 5.13: Relationship between size rank difference (total body length) of individuals of *Hoplostethus maculatus* and mean (\pm SE) attack rate. Where rank differences are positive, the attacker is larger than the individual being attacked.

Discussion

In this study, I collected information on spacing patterns, courtship behaviour and fight behaviour in *Hoplodactylus maculatus* both from field surveys and through in-depth behavioural observations of captive populations. A comparison of findings from the captive populations with those seen in the field enables me to evaluate how closely the behavioural observations are likely to reflect those encountered in the wild.

In the enclosures, animals were maintained at a sex ratio of 1:1, selected on the basis that this is the most common ratio observed amongst vertebrates (Bellairs, 1969) and that this was found to be the sex ratio in a nearby population of the same species (McIvor, 1972). However, at the study site the population was female-biased, with a sex ratio of approximately 2:1. Similarly, when collecting geckos for the enclosure populations I found that females occurred much more frequently at all sites. It is possible that this bias could be due to the capture technique used: whereas McIvor (1972) collected individuals from beneath rocks, I captured geckos basking in rock cracks. Schwarzkopf and Shine (1991) showed that capture technique significantly affected sex ratio estimates for the skink *Eulamprus tympanum*: estimates varied between months from male-biased to strongly female-biased when individuals were noosed, whereas the sex ratio of individuals captured in pitfall traps did not differ from 1:1. Such differences may be due to intersexual variation in activity through the year (Brown and Weatherhead, 2000; Bull et al., 1991) or differences in exposure. For example, it has been shown that female reptiles often bask more frequently whilst pregnant (Schwarzkopf and Shine, 1991; Shine, 1980), enabling them to maintain either higher temperatures (Brown and Weatherhead, 2000; Werner, 1990a) or greater thermal precision (Beuchat, 1986; Charland, 1995), both of which may help to reduce the length of gestation (Schwarzkopf and Shine, 1991). This has been demonstrated for *H. maculatus* from Otago (*H. maculatus* “Eastern Otago” (Hitchmough, 1997)), in which pregnant females select warmer retreat sites and maintain higher body temperatures than non-pregnant females and males (Rock et al., 2000; Rock et al., 2002). Furthermore, female reptiles in general often maintain higher body temperatures than males (Brown and Weatherhead, 2000; Shine et al., 2000a; Werner, 1990a). Therefore, it is possible that the technique used biased my samples in favour of females. However, similarly biased sex ratios have been observed in *H. maculatus* collected in pitfall traps at Turakirae Head (Whitaker,

1982) and several species of New Zealand skink (Gill, 1976; Porter, 1987). Therefore it remains to be determined whether this sex ratio bias is a real effect or an artefact of the collection procedure.

The density of geckos in the captive populations during both seasons fell well within the range experienced in the field. During 2001, densities were at the low end of the scale whereas during 2002 geckos were maintained at intermediate densities. The overall density of individuals at the field site was 0.47/m² (approximately 1800/acre). This is comparable to values found by McIvor (1972) for a nearby population of the same species on Quail Island, and by Whitaker (1982) for a population of *H. maculatus* at Turakirae Head, and is very high when compared with other published reports of lizard densities (McIvor, 1972, and references therein).

Courtship and Mating Behaviour

Very few mating events or possible mating events were observed across the two seasons. However, the timing of these confirms that the peak mating period for this species is from February-April. Perhaps more interestingly, these mating events occurred at all times of day. Supporting this was the observation of a pair mating in the field at 11 am on the rock face in full sunlight. A similar observation of an early morning mating event has been made in the closely related species *H. maculatus* “Eastern Otago” (Cree et al., 2003; Cree A, pers. comm.). These findings suggest that mating is opportunistic in this species and may be dependent upon temperature. *H. maculatus* has previously been described as nocturnal, with a peak activity period 2-3 h after sunset (McIvor, 1972, 1973). My observations confirm that this species forages at night; however, other activities, such as mating, evidently occur during both day and night, supporting the observation by Werner and Whitaker (1978) that *H. maculatus* is diurno-nocturnal.

Since so few matings were observed, I used courtship activity as an index of mating activity. The need to use observations of pairing behaviour for such purposes is fairly typical in studies on lizards, where observations of matings can be limited (Cooper and Vitt, 1993), and seems relatively justified, as Andrews (1985) found that in *Anolis carolinensis* the frequency of matings was proportional to the frequency of visits to males. In *H. maculatus*, peak courtship activity occurred from February-May (late summer to autumn) and September-October (spring). Courtship rate was greater during

2001 than 2002. This finding was unexpected, as during 2002 the populations were at greater densities and the minimum air temperature was also significantly higher. One possible explanation is that the change in methodology affected the likelihood of me observing these events. Although it was hoped that the installation of video cameras would eliminate any disturbance caused by the observer, it may be that some interactions were overlooked, taking place outside the camera's view. For example, the inside of shelters was not visible from the aerial camera. However, although several courtship interactions were observed within shelters, these made up a low proportion of the overall number seen. The region directly underneath the tin was also not visible. During the day, geckos were frequently found under the tin, presumably as it was a good heat source; however, at night individuals moved onto the floor of the enclosure. Thus, if this were a problem it would be expected that there would be significant variation in the number of courtships observed during a 24 h period. Contrary to this expectation, courtship rate did not vary with time of day. There was also no difference in courtship rate between months, suggesting that there may be two mating periods for this species: a main season between February and May and a second, shorter season from September-October, as occurs in the viviparous Tasmanian skink, *Niveoscincus ocellatus* (Jones et al., 1997).

Body size is often correlated with social status and reproductive success in lizards (e.g. Andrews, 1985; Gullberg et al., 1997; Manzur and Fuentes, 1979; Olsson and Madsen, 1995). In this study I used two measures of body size. The first, SVL, is the more commonly employed measure in studies. The advantage of this measure is that it provides a good indication of the relative size of an individual without being biased by predation history. However, this can also be a disadvantage, as tail condition can strongly affect mate choice and reproductive success (Fox et al., 1990; Martín and Salvador, 1993; Salvador et al., 1996; but see Fox and McCoy, 2000). Since predation at the study site appears to be at quite a high level, with 62% of individuals having regenerated tails, mate choice could be strongly influenced by total body length in this species.

For males, body size significantly affected courtship rate. When SVL was used as a measure of body size, larger males courted females significantly more often during 2001; little difference was seen during 2002, however. When total length was compared, the largest size class of males courted significantly more frequently during both seasons. This suggests that larger males (both SVL and total length) probably have higher

reproductive success, matching findings in other lizard species (e.g. Gullberg et al., 1997; Ruby, 1981). This could partly be due to increased testosterone concentrations in larger lizards, as found for *H. maculatus* “Eastern Otago” (Cree et al., 2003).

Snout-vent length did not significantly affect the likelihood that a female would be courted. This lack of mate choice by males could be explained if males are constrained to selecting females with a body size similar to or smaller than their own, as suggested by Olsson (1993) for *Lacerta agilis*. The finding that females of intermediate total body length were courted more frequently than the largest or smallest females suggests that males exert some degree of mate choice but that they do not necessarily prefer the largest females. Since body size has been shown to be correlated with age (Olsson and Madsen, 1995), one possible explanation for this finding could be that females of intermediate body size are younger than larger individuals and thus may have a higher reproductive potential, as found in the snake *Vipera aspis* (Bonnet et al., 2000). Another unexplored possibility is that female reproductive condition may have influenced receptivity and attractiveness (Cree A, pers. comm.). Although females from this region are believed to have an annual reproductive cycle (McIvor, 1972), in certain populations of the closely related *H. maculatus* “Eastern Otago” females have a biennial reproductive cycle (Cree, 1994; Cree and Guillette, 1995). Therefore this possibility warrants further investigation in the future.

On average, each individual courted or was courted by three members of the opposite sex and no instances of mate guarding were observed, suggesting that this species is not monogamous. Furthermore, the lack of sexual dimorphism in both body and head size (see Chapter 2) suggests that both sexes experience equivalent levels of sexual selection; thus the mating system is unlikely to be polygynous or polyandrous. Consequently, it is likely that *H. maculatus* has a polygynandrous or promiscuous mating system.

Fight Behaviour

Fighting occurred throughout the year, showing that it is not associated with the mating season and contests for mates. I found no difference in fight rate between years, despite the increased density and temperature experienced during 2002. Fight rate did vary with time of day, however. During 2001, fight rates were highest between 6 am and midday and lowest from 6 pm to midnight; in contrast, during 2002 fight rates were highest from 6 pm to midnight and lowest during the day. This could be explained by

the cooler nights experienced during the first season, resulting in a shift in peak activity periods. Peak times also varied between months. However, during the majority of the main mating season (February-April), peak fight activity occurred during the day from 6 am-12 noon and minimum activity in the afternoon and evening (midday-midnight).

The SVL of individuals did not affect the likelihood that they would initiate a fight. However, when total body length was compared it was found that individuals were more likely to attack another if they were either smaller than themselves or of similar size rank. This supports previous findings that tail condition can affect social hierarchies in lizards (Fox et al., 1990; Fox et al., 1981; Fox and Rostker, 1982; Martín and Salvador, 1993).

Fights were relatively common occurrences among both males and females. Overall, males appeared to be more aggressive than females, male-male interactions occasionally ending in a bite and wrestle. In contrast, females generally only bit or chased each other. No fight ever ended in injury to either party and there was no significant difference in the rate of fighting between the sexes. In the field, multiple individuals of the same sex were found inhabiting the same region and even the same crevice. This pattern was not dependent upon month, meaning that it occurs both outside and during the mating season. Similarly, in the enclosures, shelter sites were used by multiple individuals of the same sex, both simultaneously and at different times, and although there were instances where males or females would attack other individuals entering a shelter, there were no exclusive occupants of any one area. These findings suggest that members of this species are not particularly aggressive and that neither sex is territorial.

Conclusions

Findings from this study show that *Hoplodactylus maculatus* is a gregarious species. Although aggressive interactions occur between members of both sexes, these are generally resolved without causing injury. Such encounters occur throughout the year and thus are not associated with the mating season. Body size affects both the probability that an individual will attack another, and thus their dominance, and also the courtship rate and thus potentially the reproductive success of males. The apparent lack of territoriality in this species combined with the absence of mate guarding and the finding that individuals court or are courted by several members of the opposite sex indicate that *H. maculatus* is likely to have a polygynandrous mating system.

Chapter 6: Sperm Morphology of *Hoplodactylus maculatus*

Introduction

Sperm morphology is often related to the mating system of a species. Many comparative studies have shown that species with longer sperm often have higher levels of sperm competition (e.g. Briskie et al., 1997; Dixon and Birkhead, 1997; Gage, 1994; Gomendio and Roldan, 1991; Morrow and Gage, 2000; Pitnick and Markow, 1994), as discussed in Chapter 3. In addition to this interspecific variation, in many taxa there is also a large amount of intraspecific variation in sperm morphology. In most cases this is manifested as between-male variation within a population (Morrow and Gage, 2001), potentially providing certain males with a competitive advantage over others under sperm competition. However, in some taxa, variation is observed within an individual, with two or more sperm morphs being present.

Sperm polymorphism has only previously been demonstrated in invertebrates (e.g. see review by Swallow and Wilkinson, 2002). For example, both long and short sperm are produced within the Diptera (Bircher et al., 1995; Bressac and Hauschteck-Jungen, 1996; Snook 1997; Swallow and Wilkinson, 2002) and the nematode, *Caenorhabditis elegans* (LaMunyon and Ward, 1998). In both groups, it is the long sperm that fertilise the egg. Yet more extreme, however, is the variation observed within the Lepidoptera, where both nucleate (eupyrene) fertilising sperm and anucleate (apyrene) non-fertilising sperm are produced (e.g. see reviews by Silberglied et al., 1984; Swallow and Wilkinson, 2002).

In this chapter I investigate intraspecific variation in sperm morphology in the common gecko, *Hoplodactylus maculatus*. Males of this species produce two types of sperm that vary greatly in morphology. I describe each sperm morph and compare these to variants seen in other taxa. I then discuss their possible functions.

Methods

Collection of Samples

Male *Hoplodactylus maculatus* (*H. maculatus* “Canterbury”) were collected from the Port Hills, Christchurch. Twenty-nine individuals were collected from April-June 2002 and used for both this investigation and that outlined in Chapter 7. However, since the mating season of this species is from February-May (MacAvoy, 1976; pers. obs. Chapter 5), this was not the optimal time of year for collecting sperm. Consequently, a further six males were collected from February-April 2003. Sperm samples were collected from males through manual palpation of the lower abdomen. This resulted in a small drop of semen being squeezed from the cloaca. This was collected in a 1.1-1.2 mm capillary tube and placed on a glass slide. A coverslip was immediately added and samples were viewed with a Leica DMR microscope at a magnification of x400 with phase-contrast illumination. Samples containing sperm were recorded using a JVC TK-C1381 colour digital video camera attached to the microscope.

General Morphology

To measure sperm length, still digital images were captured from the recordings using EthoVision Color-Pro, Version 3.0.8 and sperm length was measured using Image-Pro Plus, Version 4.5.0.19. Where possible, 10 sperm of each morph were measured per male. However, this was not always feasible either due to a lack of sperm or difficulty in capturing an appropriate image. The latter was especially a problem with the smaller sperm which were highly motile, making it very difficult to capture an image containing the complete sperm. Consequently, sperm length measures for these sperm are approximations.

To further investigate the morphology of the two sperm morphs, sperm samples were viewed with scanning electron microscopy (SEM). Since several processing steps are required to prepare samples for SEM, it was necessary to adhere sperm to glass coverslips so that solutions could be changed with minimal sperm loss. Glass coverslips were placed in a subbing solution of 0.1% gelatin and 0.01% chromic potassium sulphate which had been filtered through Whatman Number 1 filter paper. Subbed coverslips were attached to glass slides using red dental wax, and a wax well was created on each coverslip to retain sperm samples within a defined region. Sperm samples had been

prefixed in 2.5% glutaraldehyde, 1% paraformaldehyde and 0.1M sodium cacodylate at a pH of 7.4. Several drops of sperm were placed in each well and left in a humidifier for 2 h, to allow sperm to settle on and adhere to the sticky coverslips. The fixative was then gently pipetted from the well and replaced with 0.1M sodium cacodylate buffer. Samples remained in the buffer for a further 2 h, following which they were dehydrated through an ethanol series (30%, 50%, 70%, 80%, 90%, 100%) for a minimum of 1 h at each concentration. They were then left in fresh absolute ethanol overnight in a humidifier. Following dehydration, samples were placed in a vacuum desiccator. The wax was then removed and coverslips were mounted on aluminium SEM stubs using conductive carbon paint and sputter-coated with ca. 40 nm gold/palladium. Samples were viewed using a Leica S440 scanning electron microscope at accelerating voltages of 15 kV.

Motility

As part of an investigation into the effect of temperature on sperm motility (outlined in Chapter 7), sperm samples were collected from twenty-nine males captured from April-June 2002. To avoid confounding any assessment of motility of the two sperm morphs with temperature, only samples collected from males held at 25°C were used in motility comparisons. Males were held in small glass aquaria (30 x 15 x 15 cm deep) in a 25°C constant temperature room on a 12 h light:dark cycle. Samples were taken from males that had been held in captivity for between 1 and 23 days. The proportion of motile sperm in the two size classes was compared across samples and, more conservatively, across males (eliminating pseudoreplication within males).

DNA Content

To determine the DNA content of the two sperm morphs, two different DNA stains were used: Hoescht 33342 (concentration = 450 µg/ml) and ethidium bromide (0.05-0.5 mg/ml). Samples were viewed under a Zeiss Axioskop 2 MOT epifluorescent microscope at a magnification of x400. Staining properties were assessed under DAPI (Hoescht 33342) and UV (ethidium bromide) illumination. Images were captured using a Zeiss AxioCam HRc CCD camera and AxioVision 3.1 software.

Results

General Morphology

Hoplodactylus maculatus males produce two sperm morphs, ‘long’ and ‘short’ (Fig. 6.1). Both morphs were present in males sampled from February to June. The long morph is over twice the length of the short morph (mean total length: long = $70.85 \pm 0.72 \mu\text{m}$; short = $30.11 \pm 1.45 \mu\text{m}$). This difference is due to a reduction in both head and tail length in the short morph. There was no significant difference in the length of the long morph between individuals (ANOVA: $F_{7,39} = 0.64$, $P = 0.72$). However, the short morph varied considerably in length between males ($F_{12,113} = 9.28$, $P < 0.001$). The length of the long morph was negatively correlated with snout-vent length ($F_{1,6} = 10.69$, $r^2 = 0.64$, $P = 0.02$), whereas the short morph did not vary with body size ($F_{1,11} = 0.63$, $r^2 = 0.05$, $P = 0.44$).

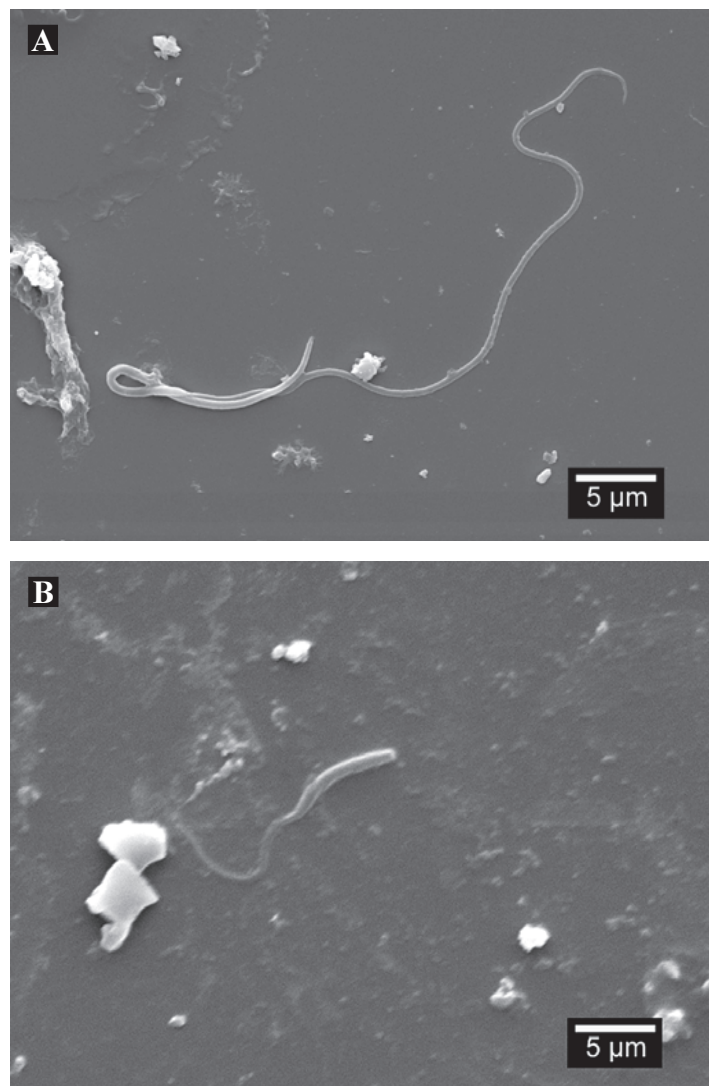


Fig. 6.1: SEM micrographs of A. ‘long’ and B. ‘short’ sperm morphs from *Hoplodactylus maculatus*.

As well as differing in length, the morphology of the two sperm morphs also differs in other aspects. The short morph has a short, rounded head which lies in line with the tail, whereas the long morph has an elongated head region which was often observed bent back upon itself (Fig. 6.2).

Both sperm morphs were found not only in the same male ($n = 4$), but also in the same sample ($n = 6$). Overall, however, the short sperm morph was more common than the long morph, occurring in 73% of all samples collected (small sperm: 70%; long sperm: 18%; short and long sperm: 12%; $n = 50$). A similar bias was observed across males when samples from an individual were combined (short: 62.5%; long: 12.5%; long and short: 25%; $n = 16$).

Motility

The motility of the two sperm morphs varies considerably. Whereas a high proportion of samples with short sperm contained at least some that were motile, the majority of long sperm samples contained immotile sperm (motility across samples - short sperm: 95.2%, $n = 42$; long sperm: 26.7%, $n = 15$). A similar pattern was observed when

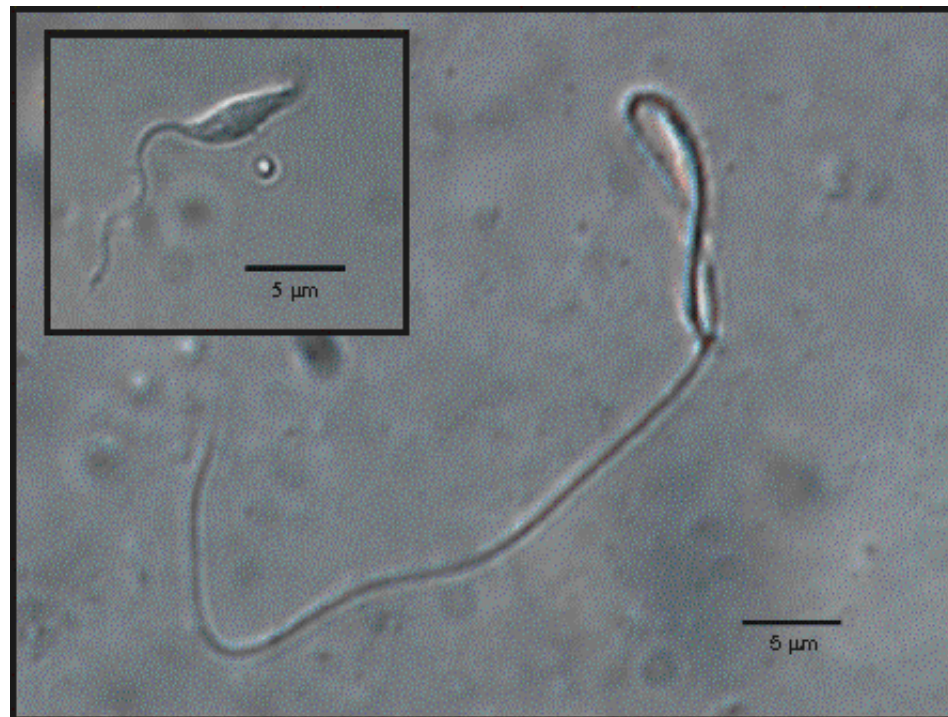


Fig. 6.2: Light micrographs of ‘long’ and ‘short’ (inset) sperm morphs of *Hoplodactylus maculatus*. Note the bent head of the long morph.

samples from an individual were combined to gain a profile for each male. Of those males that produced short sperm ($n = 14$), 92.9% produced some motile sperm; in contrast, of the males that produced long sperm ($n = 6$), only 33.3% produced any samples containing motile sperm.

The lack of motility of long sperm could be explained if motile and immotile sperm differ in morphology in some way. However, for the three males that produced both long motile and immotile sperm there was no significant difference in total sperm length between the two sperm categories (ANOVA, male = block: $F_{1,48} = 0.01$, $P = 0.91$).

A second possibility is that some other factor is required to activate motility in long sperm. As a trial, I smeared the cloacal secretions from a female onto a glass slide and added a sperm sample from a male who had only ever produced immotile long sperm. I found that a small number of sperm in the sample were active. I then added further cloacal secretions to the sample and observed a marked increase in activity. However, these results were not repeatable for a subsequent sample and remain as preliminary observations that require substantiation.

The pattern of movement also differs greatly between the two sperm morphs. Whereas motile long sperm tend to move unidirectionally, the short sperm make much less progress and were generally observed swimming in relatively tight circles.

DNA Content

Upon addition of the DNA stains Hoescht 33342 and ethidium bromide, the head of the long sperm morph fluoresced brightly (Figs. 6.3A & 6.4A). This result was found in all trials (Hoescht: $n = 5$; ethidium bromide: $n = 2$). However, the short sperm morph was never seen to fluoresce (Hoescht: $n = 3$; ethidium bromide: $n = 2$) (Figs. 6.3B & 6.4B). Large numbers of protozoan parasites inhabit the hind gut of *H. maculatus* (Hardy, 1972; Percival, 1941). Therefore, since reptiles only have one external opening for both excretory and reproductive products (the cloaca), it was fairly common to find protozoa in the sperm samples. These were seen to fluoresce with both DNA stains, demonstrating that the lack of fluorescence of small sperm was not due to inadequate staining (Fig. 6.4B).

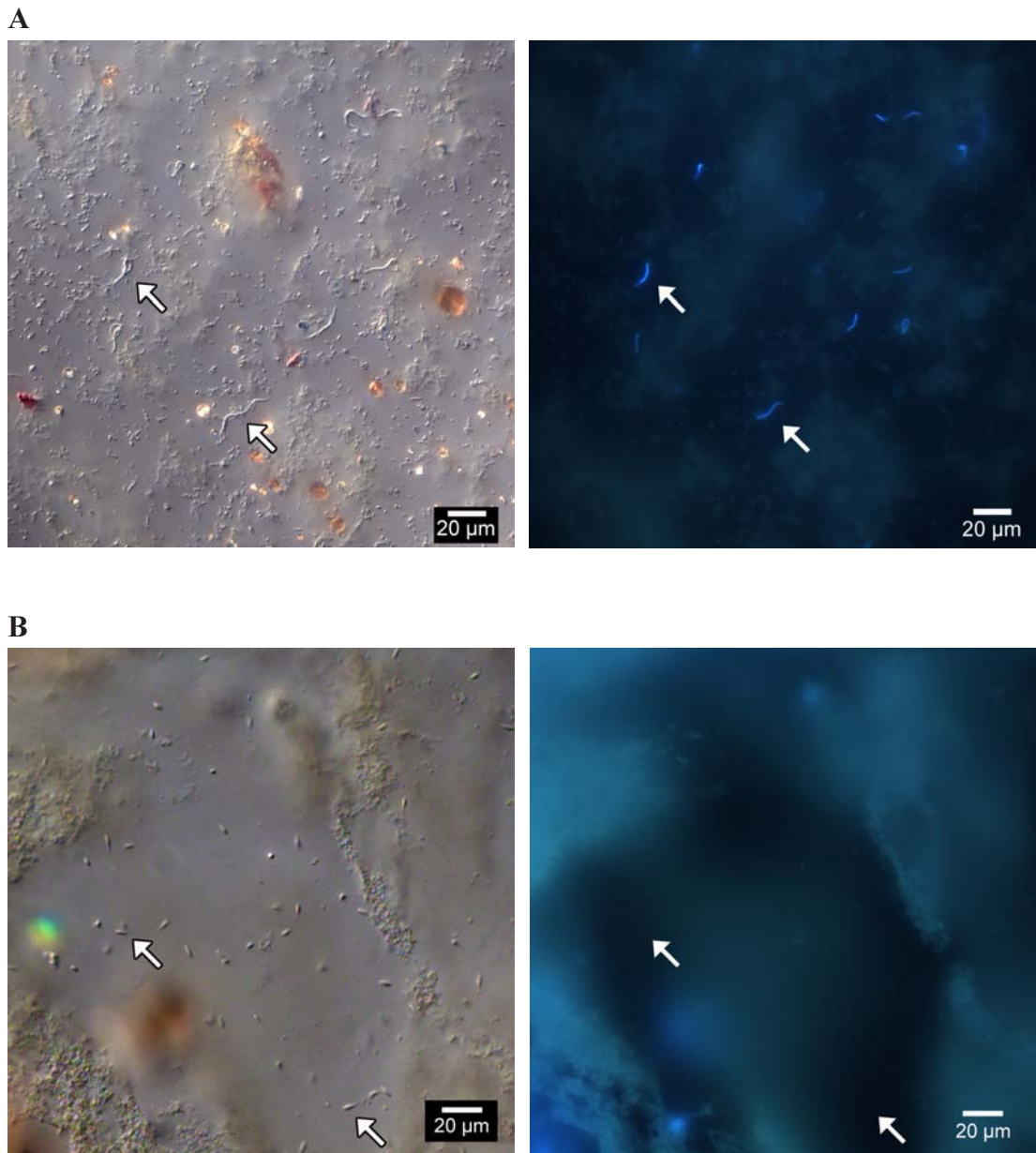


Fig. 6.3: Light micrographs of A. 'long' and B. 'short' sperm morphs from *Hoplodactylus maculatus* stained with Hoescht 33342, viewed with differential interference contrast (left) and DAPI illumination (right). Stained DNA fluoresces blue under DAPI illumination. Arrows indicate the location of two sperm.

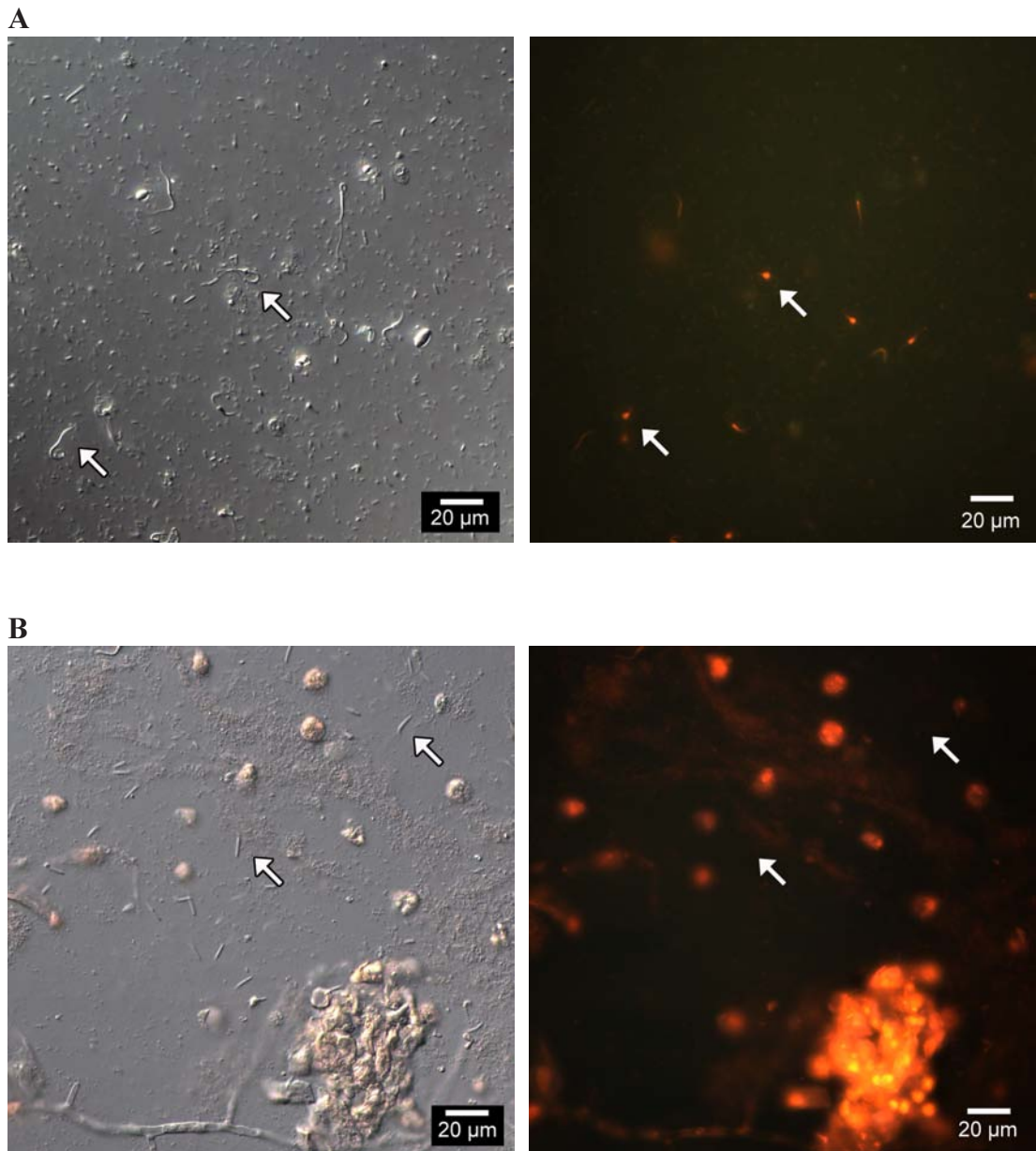


Fig. 6.4: Light micrographs of A. 'long' and B. 'short' sperm morphs from *Hoplodactylus maculatus* stained with ethidium bromide, viewed with differential interference contrast (left) and UV illumination (right). Stained DNA fluoresces orange under UV illumination. Arrows indicate the location of two sperm. Note the bright staining of protozoa but the lack of staining of short sperm (B).

Discussion

Males of *Hoplodactylus maculatus* produce two discrete sperm morphs, ‘long’ and ‘short’. The marked difference in morphology of the two sperm coupled with the observation that short sperm are present during the mating season (February-May (MacAvoy, 1973; pers. obs.; Chapter 5)) argues against the possibility that short sperm are simply degrading sperm in the male ducts. Furthermore, since short sperm were present in some individuals within 24 h of collection from the field, they are very unlikely to be a byproduct of captivity-related stress. The two morphs not only vary in length, but also in orientation of the head, many sperm belonging to the long morph having their head bent back. Newton and Trauth (1992) observed a similar bending of the head in sperm collected from the epididymis of the lizard *Cnemidophorus sexlineatus*, but showed that sperm flushed from the female oviduct no longer had this morphology. This led them to suggest that sperm may complete maturation upon entering the female reproductive tract, potentially an adaptation to prevent premature acrosomal reactions during storage in the male. Sperm of the two morphs also differ in motility, with a much greater proportion of long sperm being immotile than short sperm. This, combined with the observation that the addition of female cloacal secretions appears to increase motility, lends further support to the idea that long sperm may not complete maturation until they enter the female reproductive tract. The pattern of movement of motile sperm also differs, with long sperm moving directionally and short sperm swimming in circles. Most interestingly of all, however, is the finding that the two morphs differ in DNA content. This is the first example of a vertebrate producing a sperm morph that lacks DNA and thus that can have no direct role in the fertilisation of the egg.

Characteristics of the two sperm morphs have much in common with those seen in the Lepidoptera, the main group in which males produce non-fertilising sperm that lack DNA. In *H. maculatus*, the long fertilising sperm are more than twice the length of the short non-fertilising sperm; similarly, in Lepidoptera, the apyrene (non-fertilising) sperm are generally much shorter than the eupyrene (fertilising) sperm (Gage, 1994; Katsuno, 1978; Mancini and Dolder, 2001; Phillips, 1971; Silberglied et al., 1984; Wedell, 2001). The proportion of sperm produced also shows similarities: in the Lepidoptera, over 90% of an ejaculate may be composed of apyrene sperm (He et al., 1995; Silberglied et al., 1984; Swallow and Wilkinson, 2002); in *H. maculatus*, 70% of samples contained

only non-fertilising sperm. It should be noted that some caution is required when interpreting the results on proportional representation of the two sperm morphs in this study, as the method used to collect samples did not result in 'natural' ejaculations: droplets of semen were simply squeezed from the vas deferens or epididymis. Consequently, it is quite feasible that the composition of an ejaculate would be different had the male been mating with a female. However, the fact that there is a far greater representation of non-fertilising sperm in storage in the vas deferens / epididymis suggests that these are also likely to be transferred in much greater quantity. Finally, upon ejaculation, the apyrene sperm of Lepidoptera are motile whereas eupyrene sperm are transferred to the spermatophore in an inactive state (Garvey et al., 2000; Osanai et al., 1987; Silberglied et al., 1984); similarly, in *H. maculatus* the majority of fertilising sperm are immotile, perhaps suggesting that an activation factor is required. It is possible that this could be supplied either by the male, through the addition of seminal products (Cuellar et al. 1972; Depeiges and Dacheux, 1984; Mann, 1954; Nirmal and Rai, 1997), or by chemicals in the female reproductive tract (Mann, 1954; Newton and Trauth, 1992). Preliminary observations suggest that the latter could be the case, but more research is required before conclusions can be drawn.

Since there is such great similarity in morphology and physiology of the two sperm morphs, it is likely that there is also similarity in function. The exact function of non-fertilising sperm is still uncertain and has been reviewed by Swallow and Wilkinson (2002). It was originally thought that these non-fertilising sperm morphs may simply be aberrant sperm formed during meiosis with no adaptive function (Cohen, 1967, 1973; Wolf et al., 1987). However, in the Lepidoptera apyrene sperm are formed via a distinct developmental pathway (Friedländer and Gitay, 1972; Garvey et al., 2000; Lai-Fook, 1982) and in vast numbers (Gage and Cook, 1994; Silberglied et al., 1984), making this unlikely. Moreover, Gage and Cook (1994) found that male Indian meal moths (*Plodia interpunctella*) under nutritional stress still produced ejaculates composed of approximately 90% apyrene sperm, suggesting that they play some critical role.

One suggested adaptive function is the facilitation of transport of fertilising sperm (Buckland-Nicks et al., 1982; Friedländer and Gitay, 1972; Holt and North, 1970; Oppliger et al., 2003). Here it is argued that the fact that apyrene sperm are motile upon leaving the testes renders them capable of aiding the immotile eupyrene sperm either in

exiting the testes or passing up the female reproductive tract. However, studies to date have failed to show a close association between eupyrene and apyrene sperm (Silberglie et al., 1984). In addition, the fact that their flagella beat at different wave lengths and frequencies (Osana et al., 1987) has led to the conclusion that such facilitation is unlikely (Silberglie et al., 1984). This also seems an unlikely function in *H. maculatus*, as the short, non-fertilising sperm generally swim in tight circles, suggesting that they would not be particularly efficient in the transport of long sperm up the female reproductive tract.

The observation that non-fertilising sperm often break down within or disappear from the sperm storage sites of females (Katsuno 1977) has led to the suggestion that they may be a nutritional source either for fertilising sperm, the female or the zygote (Silberglie et al., 1984; Swallow and Wilkinson, 2002; Watanabe et al., 2000). However, it has been counter-argued that these small sperm would provide very few nutrients, especially when compared with the nutrient content of the seminal fluid (Bissoondath and Wiklund, 1996; Boggs and Watt, 1981; Cook and Gage, 1995; Silberglie et al., 1984), and Snook and Markow (1996) showed that the short non-fertilising sperm of *Drosophila pseudoobscura* do not contribute nutrients to the female.

In the silkworm, *Bombyx mori*, it was noted that apyrene sperm did not advance whilst swimming despite strong flagellar movements, leading to the idea that they may help to stir the contents of the sperm storage site resulting in dissociation of eupyrene sperm bundles (Osana et al., 1987). This swimming pattern is very similar to that seen in *H. maculatus*; however, in this species the fertilising sperm are also released individually and thus would not be expected to require mixing.

Finally, it has been proposed that non-fertilising sperm play a role in sperm competition, either destroying the sperm of rival males or serving as ‘cheap filler’ for sperm storage sites (Silberglie et al., 1984). The former of these ideas has led to much speculation and the suggestion that there may be a similar partitioning of roles among sperm within mammalian ejaculates, where certain classes of sperm may take on ‘kamikaze’ (Baker and Bellis, 1988, 1989; Bellis et al., 1990) or ‘soldier sperm’ (Kura and Nakashima, 2000) roles, either seeking and destroying or blocking the sperm of rival males. However, persuasive evidence for this is still lacking (see Harcourt, 1989, 1991). In contrast, there is some evidence that in Lepidoptera non-fertilising sperm

may act as ‘cheap fillers’, since in some species females storing larger quantities of apyrene sperm have a greater remating interval (Cook and Wedell, 1999; He et al., 1995; Wedell, 2001). Sperm competition hypotheses also seem to provide the most likely explanation for the role of non-fertilising sperm in *H. maculatus*. The fact that the short sperm swim in circles and do not appear to advance far may suggest they play a role in preventing the sperm of rival males from advancing through the vagina. Alternatively, since females of this species have sperm storage sites (see Chapter 4), it is also quite feasible that if these sperm reach the storage sites they may act as fillers, preventing rival sperm from entering.

The close similarities between the two sperm morphs observed in *H. maculatus* and those observed in many invertebrates, particularly the Lepidoptera, render it possible to utilise and build upon theories proposed for the latter group. The composition of ‘natural’ ejaculates and the exact function of the non-fertilising sperm remain to be determined, providing a multitude of research possibilities for this species in the future.

Chapter 7: Effect of Temperature on Sperm Motility in *Hoplodactylus maculatus*

Introduction

Temperature can have a large effect on both physiological and behavioural processes in animals. Although reptiles are ectotherms, they are able to exert a relatively large amount of control over their body temperatures, keeping them within a relatively narrow range during the day (Pough, 1980). This can be achieved physiologically, for example through panting and evaporation (Stevenson, 1985) and by altering features such as blood flow (Bartholomew and Tucker, 1963, 1964), metabolic rate (Bartholomew and Tucker, 1963, 1964; Richards, 1973) and skin colour (Bradshaw and Main, 1968; Heatwole, 1970). Perhaps more important, however, is behavioural thermoregulation, achieved through basking and shuttling between sun and shade (Avery, 1979; Bogert, 1949; Stevenson, 1985; Werner, 1990b), changing body postures in the sun (Avery, 1979; Bradshaw and Main, 1968; Heatwole, 1970), conduction of heat from objects such as rocks (Avery, 1979; Bogert, 1949; Bustard, 1967; Dial, 1978; Rock et al., 2002) and social grouping (Stevenson, 1985). The latitude at which a species lives has a large influence on the relative importance of these thermoregulatory mechanisms: species inhabiting temperate zone environments will need to spend a much greater amount of time thermoregulating than tropical species (Shine and Madsen, 1996). The most important factor affecting behavioural thermoregulation in reptiles is the time of activity, both on a seasonal and daily basis (Stevenson, 1985).

Whereas diurnal species are able to regulate their body temperatures within a relatively narrow range, maintaining temperatures close to optimal, nocturnal species have far fewer opportunities to thermoregulate. Temperature can still be controlled to some extent through the selection of warmer retreat sites (Avery, 1982; Stevenson, 1985; Werner, 1990b), but on the whole is dictated by and strongly correlated with ambient air temperature (Avery, 1979; Licht et al., 1966; Parker and Pianka, 1974; Pianka and Huey, 1978; Pianka and Pianka, 1976; Werner, 1990b; Werner and Whitaker, 1978). Consequently, nocturnal reptiles have their peak activity periods during a time when their body temperatures are sub-optimal (Angilletta and Werner, 1998; Dial, 1978; Huey et al., 1989; Huey and Slatkin, 1976). To counter this, many nocturnal species are only active for the first few hours following sunset (Avery, 1979, 1982), when their

body temperatures are still above ambient temperature. In addition, it has been shown that lizards tend to heat more rapidly than they cool (Bartholomew, 1982; Bartholomew and Tucker, 1963, 1964; Richards, 1973; but see Arad et al., 1997), allowing higher body temperatures to be maintained for longer. However, for those species that are active for extended periods of the night, large variations in temperature will be experienced (Dial, 1978).

Although activity occurs at lower temperatures in nocturnal species, it has been shown that preferred or optimal body temperatures are similar to those seen in diurnal species (Bustard, 1967; Huey et al., 1989). Consequently, a bimodal temperature profile is often observed in these species (Angilletta and Werner, 1998; Bustard, 1967; Huey, 1982), with many species still thermoregulating to some extent during the day (Avery, 1982; Bustard, 1967; Huey and Slatkin, 1976; Rock et al. 2000; Rock et al., 2002; Schlesinger and Shine, 1994; Werner, 1990b; Werner and Whitaker, 1978). This allows the temporal separation of activity from other key functions (Angilletta et al., 1999), allowing physiological processes, such as digestion, to occur at optimal temperatures (Bustard, 1968a; Dial, 1978). Such decoupling of processes is not always possible, however. In particular, reproductive activity will often be constrained to periods of peak activity, meaning that associated physiological functions may be operating in sub-optimal conditions.

Since temperature affects the activity profiles of reptiles (Bustard, 1967, 1968b), it will also influence reproductive activity which is likely to be constrained to certain times of the year and day in nocturnal species. In turn, physiological processes linked to reproductive success are likely to be affected. The interaction between temperature and reproductive cycles of male and female lizards has been well investigated (Cowles and Burleson, 1945; Crews, 1975; Licht, 1984). However, few studies have investigated the direct effect of temperature on short-term reproductive physiology, such as sperm viability. High temperature has been shown to cause an increased incidence of abnormal sperm or sterility in several taxa (Ashizawa et al., 1998; Perez-Velazquez et al., 2001) and has been suggested as a possible reason for the externalisation of testes in mammals (Bryden, 1967; but also see Chance, 1996; Freeman, 1990) and the sperm storage organ in birds and mammals (Freeman, 1990). A similar link between high temperature and damage of testicular material or sterility has been shown to occur in lizards (Cowles and Burleson, 1945; Licht, 1965; Licht and Basu, 1967). At the other end of the

temperature scale, it has been found that colder temperatures result in lower tail beat frequencies in sperm (Billard and Cosson, 1992); swimming speed has, in turn, been linked with fertilisation success (Birkhead et al., 1999; Levitan, 2000; but see Aas et al., 1991). There is also often a trade-off between swimming speed and duration of motility, with sperm being motile for longer at cooler temperatures (Billard and Cosson, 1992; Levitan, 2000; Vladic and Järvi, 1997). To date, the relationship between temperature and sperm swimming speed and duration of motility has only been investigated in species with external fertilisation. However, it is likely that such an interaction between temperature and sperm viability is also important in ectothermic species with internal fertilisation, such as reptiles. This is especially likely to be the case in nocturnal species, where mating often occurs at low body temperatures.

New Zealand geckos of the genus *Hoplodactylus* are good experimental subjects for an investigation into the interaction between temperature and sperm motility, as not only do they inhabit a temperate environment subject to wide temperature fluctuations on a seasonal and daily basis, but they have been shown to be active and forage at particularly low temperatures compared with other nocturnal geckos (McIvor, 1973; Rock et al., 2002; Werner and Whitaker, 1978). Furthermore, although some reproductive activities occur during the day, reproductive behaviour also occurs at night (pers. obs.; Chapter 5), when temperatures are unlikely to be optimal for such activities. In this chapter I determine the relationship between body temperature and sperm motility in the common gecko, *H. maculatus*. These results are used to make predictions about the likely impact of temperature on reproductive success in this species with respect to male-male competition and body size.

Methods

Collection and Recording of Sperm

Twenty-nine male *Hoplodactylus maculatus* (*H. maculatus* “Canterbury”) were collected from the Port Hills, Christchurch from April-June 2002. Although this was not the optimal time for sampling sperm, as it is at the end of the mating season (MacAvoy, 1973; pers. obs. Chapter 5), there was no evidence of degeneration of sperm during this period. Only mature individuals of snout-vent length greater than 60 mm were collected. Animals were returned to the laboratory and placed individually into small (30 x 15 x 15 cm deep) glass aquaria, containing a substrate of gravel and loose bark, and branches

as shelter and perching sites. Water was available at all times and individuals were fed house flies once a week. The aquaria were kept in a 25°C constant temperature room on a 12 h light:dark cycle.

To test the effect of temperature on sperm motility, males were placed in constant temperature rooms held at three different temperatures: 5°C (mean = $5.7 \pm 0.10^\circ\text{C}$), 15°C (mean = $15.3 \pm 0.09^\circ\text{C}$) and 25°C (mean = $24.4 \pm 0.12^\circ\text{C}$). All males experienced all three temperatures in a random order. Between trials, animals were returned to the 25°C room overnight, as this was considered the optimal temperature available for males to replenish their sperm supplies.

Males were placed at the appropriate temperature early in the morning. They were then given an acclimatisation period, the length of which depended upon the temperature being tested: 25°C - no acclimatisation required as held overnight at this temperature; 15°C - minimum period before testing = 6 h; 5°C - 2 h at 15°C followed by a minimum period of 6 h at 5°C. Trials showed that these times were sufficient for the cloacal temperature of males to be reduced to within 5°C of the target temperature. Since males housed at 25°C were able to maintain their cloacal temperatures at up to 5°C higher than ambient it was felt that this represented acclimatisation. Due to the different lengths of acclimatisation required, for practical reasons males held at 25°C were tested first, followed by those at 15°C then 5°C. Although this meant that there was a fixed temporal order to trials at each temperature, it was considered that this should have minimal impact on the results as there is no reason to assume that sperm motility should vary with time of day. All equipment used to collect and record sperm motility was also held at ambient temperature for a minimum of 1 h; sperm motility was recorded in the constant temperature rooms to avoid the possibility that sperm might change temperature and thus motility during trials.

Sperm samples were collected from males through manual palpation of the abdomen. Latex gloves that had been held at the appropriate temperature were worn to reduce the probability of my hands warming the gecko. The droplet of semen produced was collected in a 1.1-1.2 mm diameter capillary tube and placed on a glass slide. The sample was then immediately covered with a glass coverslip, placed under the microscope and viewed at a magnification of x400 with phase-contrast illumination. A Leica DMR microscope was used, to which a JVC TK-C1381 colour digital video camera had been attached. This was linked to a VCR. Each sperm was recorded for a minimum of 10

sec and samples were recorded for up to 10 min. Up to four samples were obtained from each male using the above procedure as not all samples contained free-swimming sperm. Following trials, all males were returned to their collection sites. On average, each male was held in captivity for three weeks; any males that did not produce sperm after two weeks of trials were returned to the field.

Long vs Short Sperm

H. maculatus males produce two sperm morphs: long fertilising and short non-fertilising (see Chapter 6). I found that very few samples collected contained long sperm (see Results). Consequently, I tested whether temperature affected the probability that long sperm were present in a sample. The presence/absence of long sperm was recorded for all samples collected from males. Since sample number could affect the likelihood of obtaining long sperm, i.e. they could be provided earlier/later in an ejaculate, sample number (of those containing sperm) was included as a factor in the analysis.

Sperm Motility

Since not all sperm in a sample were motile, I first analysed the effect of temperature on the proportion of sperm motile. All samples from all males tested were analysed and the number of sperm motile and immotile recorded. Since it is possible that sample number could have an effect on the proportion of sperm motile, e.g. sperm stored deeper in the epididymis could be more motile than those closer to the cloaca, sample number (of those in which sperm were collected) was included as a factor in the analysis. In addition, samples were separated into 1 min blocks and time was included as a factor in the analysis, to account for any temporal variation in motility.

Of those sperm that were motile, tail beat frequencies were used as an index of sperm motility. Samples were analysed using iMovie Version 2.1.2. Only males that had provided sperm at all three temperatures were included in the analysis. Up to 10 free-swimming sperm were selected from one sample for each male. For each sperm I recorded the number of full tail beats over a 10 sec period. Where possible, the first sample collected was used, to minimise the risk that I was warming the geckos over time. However, since the first sample did not always contain sperm or suitable sperm for analysis, a record was made of sample number (including those in which sperm were not collected) and sample was included as a factor in the analysis. Similarly, each

sperm analysed was taken from as early in the sample as possible. However, since there was a large amount of variability in times between sperm, time was included as a covariate in the analysis.

Data Analysis

Where multiple samples were obtained for each male at each temperature, split-plot ANOVA/ANCOVA was used to determine the effect of temperature on sperm characteristics. In these analyses, male was included as a block, temperature as a plot, sample as a subplot and time as a factor or covariate nested within sample. Where the response was categorised as presence/absence or a proportion, binomial tests were used. However, where data did not meet expectations of the binomial distribution, i.e. residual deviance did not equal residual degrees of freedom, an F-test was used as an approximation.

Results

Long vs Short Sperm

In total I collected sperm samples from 18 males. However, only nine of these produced long sperm in one or more samples. Split-plot ANOVA with a binomial response (presence/absence of long sperm) showed that there was a significant difference between temperatures in the likelihood of long sperm being present in a sample ($F_{2,28} = 12.60$, $P < 0.001$), with a greater proportion of samples collected at higher temperatures containing long sperm ($25^{\circ}\text{C} = 0.29$; $15^{\circ}\text{C} = 0.19$; $5^{\circ}\text{C} = 0.09$). Sample number had no influence on the presence of long sperm ($F_{3,104} = 1.09$, $P = 0.36$) and there was no interaction between temperature and sample ($F_{5,104} = 0.43$, $P = 0.83$).

Sperm Motility

Since so few long sperm samples were obtained it was not possible to determine the effect of temperature on sperm motility for these fertilising sperm. However, I did find that there was no significant difference between the motility of long and short sperm at 25°C (split-plot ANOVA with male as source of replication: $F_{1,13} = 1.79$, $P = 0.20$). Time had a significant effect on the motility of sperm ($F_{1,116} = 8.89$, $P = 0.003$), with motility decreasing as time increased (Fig. 7.1). There was no interaction between

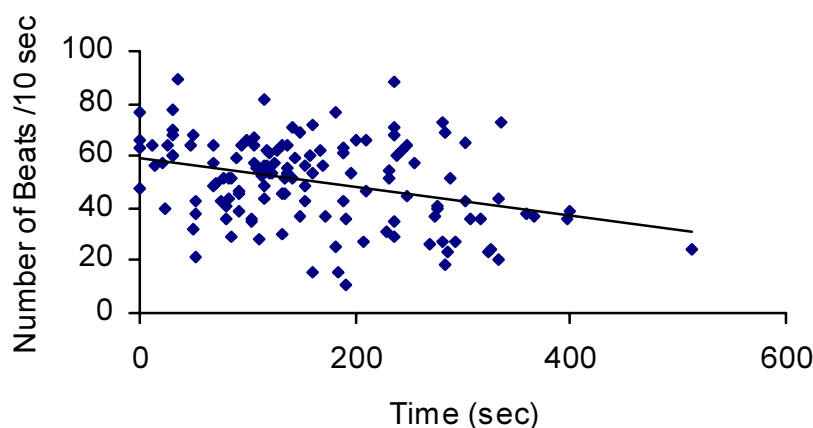


Fig. 7.1: Relationship between sperm tail beat frequency (number over 10 sec) and time in samples of long and short sperm collected from *Hoplodactylus maculatus* held at 25°C .

sperm length and time ($F_{1,116} = 0.007$, $P = 0.93$), meaning that this reduction in motility through time occurred for both sperm morphs.

Thirteen males provided samples containing short sperm. Although most samples contained some motile sperm, the proportion varied from 0 to 1 (mean = 0.41 ± 0.01). Split-plot ANCOVA with a binomial response (proportion of motile and non-motile sperm) showed that temperature had a significant effect on the proportion of sperm motile in a sample (Table 7.1), higher temperatures being associated with a greater proportion of motile sperm (Fig. 7.2). There was a significant difference in motility between all three temperatures (25 x 5°C: $t_{283} = 19.58$, $P < 0.001$; 25 x 15°C: $t_{335} = 8.46$, $P < 0.001$; 15 x 5°C: $t_{374} = 10.51$, $P < 0.001$). The proportion of motile sperm also varied significantly between samples, with both samples 1 and 4 containing significantly more motile sperm than samples 2 and 3 (1 x 2: $t_{292} = 15.60$, $P < 0.001$; 1 x 3: $t_{112} = 12.55$, $P < 0.001$; 4 x 2: $t_{12} = 3.97$, $0.001 < P < 0.002$; 4 x 3: $t_{13} = 3.58$, $0.002 < P < 0.005$) (Fig. 7.3). No other main effects or interactions were significant. The total number of samples collected varied between males. Thus sample size decreased with increasing sample number, and only three males produced four samples, on only one occasion each. Analysis of the proportion of sperm motile in each sample for these males showed no consistent order effect (Male 1 (15°C): Sample 3>4>1>2; Male 2 (5°C): Sample 2>1>4>3; Male 3 (5°C): Sample 3>2>4>1).

Samples containing the short sperm morph were collected at all three temperatures for 13 males. I found that temperature significantly affected sperm motility (sqrt transformed) (Table 7.2), with sperm collected at higher temperatures beating faster than those at lower temperatures (Fig. 7.4). There was a significant difference in tail

Table 7.1: Effect of temperature, sample number and time on the proportion of motile short sperm in samples from *Hoplodactylus maculatus*. Split-plot ANCOVA; binomial response (* $P < 0.05$).

Source	df	Deviance	Error term	F	P
Male	12	1706.90	Male x Temp	3.27	0.008*
Temp	2	414.80	Male x Temp	4.77	0.02*
Male x Temp	22	955.80			
Sample	3	151.25	Male x Temp x Sample	3.84	0.02*
Temp x Sample	5	20.10	Male x Temp x Sample	0.31	0.91
Male x Temp x Sample	45	591.30			
Time	9	40.59	Residual	1.40	0.19
Temp x Time	17	72.83	Residual	1.33	0.17
Sample x Time	22	40.26	Residual	0.57	0.94
Temp x Sample x Time	29	133.20	Residual	1.43	0.07
Residual	401	1290.90			

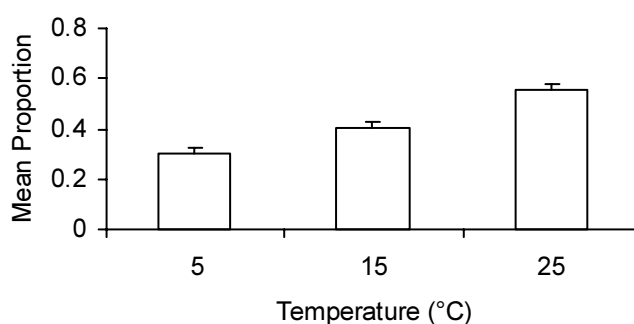


Fig. 7.2: Mean proportion (\pm SE) of motile short sperm in samples from *Hoplodactylus maculatus* held at 5, 15 and 25°C.

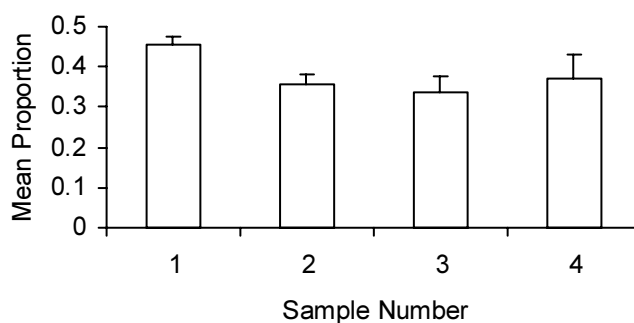


Fig. 7.3: Relationship between sample number and the mean proportion (\pm SE) of motile short sperm in samples from *Hoplodactylus maculatus*.

Table 7.2: Effect of temperature, sample number and time on tail beat frequency of short sperm in samples from *Hoplodactylus maculatus*. Split-plot ANCOVA; sqrt transformed response (* $P < 0.05$).

Source	df	Deviance	Error term	F	P
Male	12	155.8	Male x Temp	1.29	0.29
Temp	2	630.74	Male x Temp	31.34	<0.001*
Male x Temp	24	241.53			
Sample	3	9.48	Male x Temp x Sample	0.69	0.56
Temp x Sample	6	30.95	Male x Temp x Sample	1.13	0.37
Male x Temp x Sample	32	146.34			
Time	1	1.16	Residual	0.62	0.43
Temp x Time	2	14.81	Residual	3.95	0.02*
Sample x Time	3	7.37	Residual	1.31	0.27
Temp x Sample x Time	6	13.83	Residual	1.23	0.29
Residual	494	925.81			

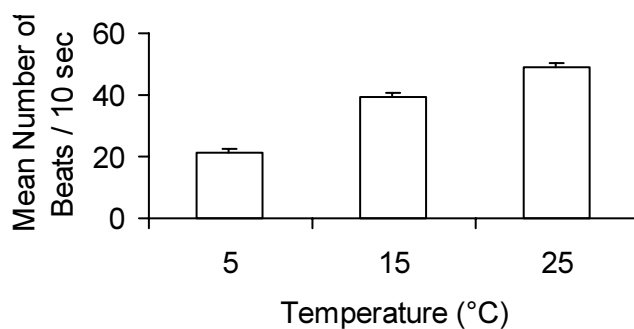


Fig. 7.4: Mean tail beat frequency (\pm SE) of short sperm in samples from *Hoplodactylus maculatus* held at 5, 15 and 25°C.

beat frequency between all three temperatures (Tukey Test - 25 x 5°C: $q_{24,3} = 11.47$, $P < 0.001$; 25 x 15°C: $q_{24,3} = 3.69$, $0.025 < P < 0.05$; 15 x 5°C: $q_{24,3} = 7.45$, $P < 0.001$). There was also a significant interaction between temperature and time, with tail beat frequency decreasing through time for samples collected at 25°C but remaining relatively constant for those at 5 and 15°C (Fig. 7.5). No other main effects or interactions were significant.

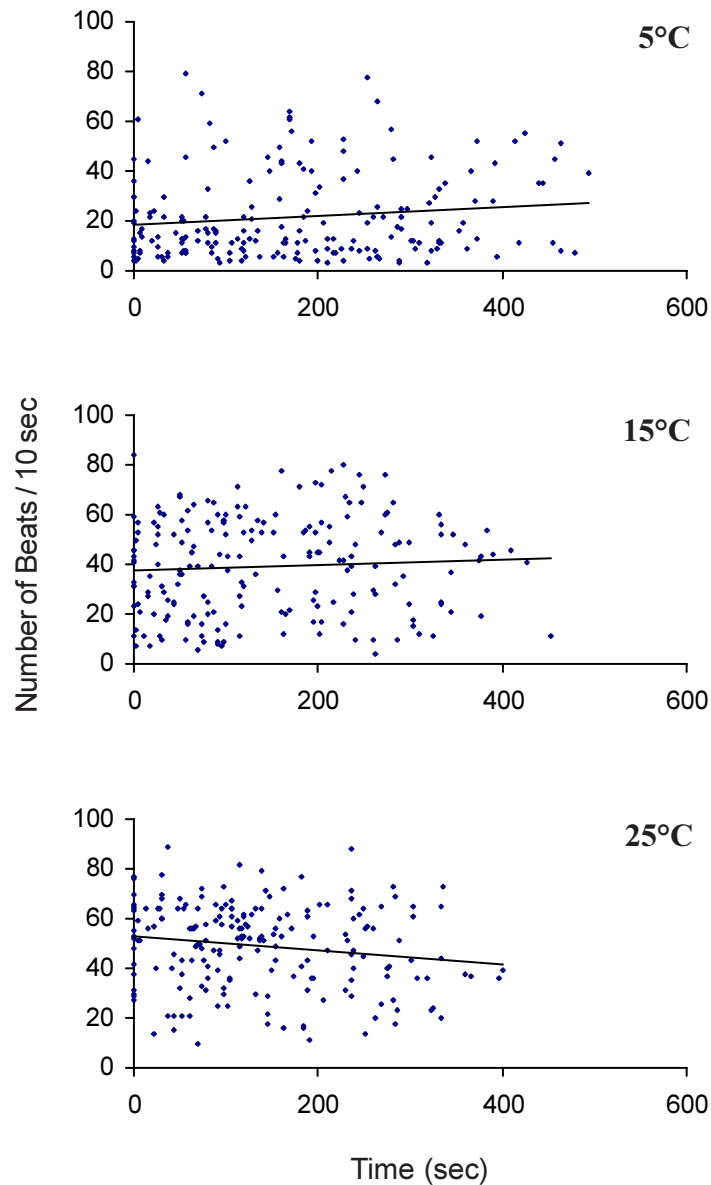


Fig. 7.5: Effect of time on motility of short sperm (number of beats over 10 sec) at 5, 15 and 25°C in samples from *Hoplodactylus maculatus*.

Discussion

Temperature had a significant effect on the likelihood of obtaining long sperm in a sample. This finding may reflect the specific location within the epididymis from which sperm samples were taken. In general, it was much easier to obtain samples from geckos held at warmer temperatures, as they appeared to be more relaxed. Consequently, larger samples were often obtained, which are likely to have contained sperm stored deeper in the epididymis. If this is the case, it suggests that long fertilising sperm are transferred later in the ejaculate than the short non-fertilising sperm. Similar differences in transfer times have been observed in other taxa. For example, Holt and North (1970) found that in the cabbage looper, *Trichoplusia ni*, the apyrene or non-fertilising sperm are found closer to the opening of the vesicle receptacle, suggesting they may be transferred first. In the small white butterfly, *Pieris rapae*, a greater proportion of eupyrene or fertilising sperm are often transferred in second matings (Cook and Wedell, 1996; Wedell and Cook, 1999). However, my finding that sample number did not significantly affect the proportion of long sperm present argues against this. A second possibility for this temperature effect is that the amount of energy required to produce long sperm is greater than that necessary for short sperm. All males were held overnight at 25°C between trials to replenish sperm supplies before resampling. However, it is possible that this was insufficient time for the production of long sperm, so that only those individuals maintained at higher temperatures for longer periods were able to replenish some of their supplies. Such a difference in maturation time has been observed in *Drosophila subobscura*, where nearly all males produce short sperm first (Bircher et al., 1995). Alternatively, it is possible that since long sperm are probably more costly to produce, males only ejaculate these at higher temperatures when they are likely to be most successful.

The finding that there was no significant difference in tail beat frequencies of long and short sperm morphs at 25°C was unusual, as it would be expected that wave lengths along the flagella would have differed due to their size difference, as found in the silkworm, *Bombyx mori*, where longer, eupyrene sperm have a longer wavelength and lower frequency of beating (Osanai et al., 1987). This lack of difference makes it likely that the two morphs would react in similar ways to changes in temperature. Thus, the temperature effects seen for short sperm are likely to be mirrored in long sperm.

There was a greater proportion of motile short sperm in samples at higher temperatures, suggesting a high degree of temperature sensitivity of these sperm. It is unknown whether immotile sperm were inactive or dead, but the net effect would be identical, as in both cases a lower proportion of sperm transferred at lower temperatures would be capable of travelling up the female oviduct and reaching sperm storage sites. The finding that sample number also had a significant effect on the proportion of motile sperm is more difficult to interpret. It was initially thought that sample number could be important as sperm located closer to the cloaca are likely to have been in storage for longer than those obtained from deeper in the epididymis. This could have either a positive or negative effect on sperm motility depending upon the physiological processes occurring in the epididymis. On the one hand it is possible that there would be a greater proportion of motile sperm in samples taken from deeper in the epididymis as these are likely to have been produced more recently. Alternatively, it is possible that sperm are activated by the addition of seminal products in the epididymis (Cuellar et al. 1972; Depeiges and Dacheux, 1984; Mann, 1954; Nirmal and Rai, 1997). If this activation occurs after a set amount of time in storage, or if activation substances are released further down the epididymis, as found in other lizards (Depeiges and Dacheux, 1984; Nirmal and Rai, 1997), it is possible that samples taken from nearer to the cloaca would contain more motile sperm. Either of these predictions would suggest that there should be a specific order effect on the proportion of motile sperm in a sample. Therefore, the finding here that both the first and last samples collected contained more motile sperm than those collected in the middle is difficult to explain and could, in fact, be a result of the methodology used when collecting samples. There was no set number of samples taken for each individual, as some geckos only produced one sample whereas others produced up to four; no more than four samples were obtained from any individual to minimise handling times and stress to the animal. Consequently, sample sizes varied greatly, decreasing with increasing sample number. The large potential effect of this variation is highlighted by the fact that when the effect of sample number on the proportion of motile sperm was analysed for the three males from which four samples were obtained, no consistent order effect was observed. In addition, it was not possible to control the volume of sperm in each sample, as a droplet of variable size was produced in each case. Therefore, although the first sample collected will always have contained

a relatively high proportion of sperm located near the cloaca, samples 2-4 are likely to have contained a mixture of sperm from near the cloaca and deeper in the epididymis. Consequently, it is likely that the greater proportion of motile sperm in the first sample was a real effect, suggesting that some activation factor may need to be added to sperm prior to transfer. However, the result for sample 4 is possibly an artefact of the methodology used.

As well as increasing the proportion of motile sperm in a sample, higher temperatures also resulted in increased tail beat frequencies in short sperm, matching findings from freshwater fish (Billard and Cosson, 1992). Moreover, at 25°C there was a concurrent decline in motility through time, whereas sperm collected at 5 and 15°C maintained the same motility throughout the sampling period. A negative correlation between temperature and sperm motility duration has also been found in other taxa (Billard and Cosson, 1992; Vladic and Järvi, 1997) and suggests that sperm contain a limited supply of energy resulting in a trade-off between speed and stamina, as found in the sea urchin, *Lytechinus variegatus* (Levitan, 2000).

Overall, the results obtained from this study suggest that temperature has a marked effect on sperm transfer and motility in *Hoplodactylus maculatus*. Not only are more long, fertilising sperm produced or transferred at higher temperatures, but temperature also affects sperm motility, both in terms of the proportion of sperm motile and tail beat frequencies of active sperm. This has important implications for the reproductive ecology of this species. Since *H. maculatus* is nocturnal, its main activity period is during a part of the day when there is little opportunity to thermoregulate. Consequently, its body temperature is likely to be very closely correlated with ambient temperature (Avery, 1979; Licht et al., 1966; Parker and Pianka, 1974; Pianka and Huey, 1978; Pianka and Pianka, 1976; Werner, 1990b; Werner and Whitaker, 1978). Although many nocturnal lizards facing such challenges restrict their activity periods to a few hours after sunset (Avery, 1979, 1982), during which time air temperatures are still relatively mild and they are able to maintain their body temperatures a few degrees above ambient, *H. maculatus* does not appear to follow this activity cycle, often being active from dusk to dawn (pers. obs.; Chapter 5). In addition, this species mates from late summer to autumn (February-May) (MacAvoy, 1976; pers. obs.), during which time night temperatures can be as low as 1°C and are rarely greater than 15°C (pers. obs.).

Consequently, it is likely that individuals will regularly experience temperatures of 5-15°C during their reproductive season. One possible method by which such low temperatures may be avoided whilst mating is through restricting copulations to during the day (Olsson and Madsen, 1998). Although it appears that some mating activity does occur during the day in this species, courtships occur throughout the 24 h period (pers. obs.; Chapter 5). Therefore, it is likely that sperm are often transferred under sub-optimal conditions.

This strong effect of temperature on sperm motility also has implications for sperm competition in this species, as males may differ in their ability to maintain their bodies at higher temperatures. One factor that can have a large influence on the body temperature of reptiles is body size. Smaller individuals have a larger surface area to volume ratio, meaning they both heat and cool more rapidly than larger individuals (Bartholomew, 1982; Bartholomew and Tucker, 1964; Cowles, 1941, 1945; Hertz et al., 1993). This effect is especially evident when comparing individuals across species that vary by orders of magnitude in body size (Stevenson, 1985), but is also important within a species, as demonstrated by the fact that juveniles and subadults are often more active than adults later in the season, as they are able to raise their body temperatures relatively quickly even though ambient temperatures are low (Cowles, 1941; Huey and Slatkin, 1976). Although in several lizard species, including *H. maculatus*, it has been demonstrated that body size has no effect on body temperature (Bogert, 1949; Heatwole, 1970; McNab and Auerberg, 1976; Walker et al., 1991; Werner and Whitaker, 1978), in all these studies temperatures were collected during the day, when individuals are able to regulate their temperatures. In contrast, at night body size is likely to be an important determinant of body temperature, as heating and cooling rates will dictate the temperature of the animal. In a nocturnal species such as *H. maculatus*, there are two possible consequences. Firstly, it is possible that smaller individuals that are able to warm up more quickly will be able to better utilise smaller variations in microclimate to maintain their body temperatures above ambient, either through the use of warm retreats or simply by having greater contact with the warmer substrate (Cowles, 1941; Stevenson, 1985). In contrast, larger individuals will cool less rapidly than smaller individuals following sunset (Bartholomew, 1982; Hertz et al., 1993). Shine et al. (2000a) recently demonstrated that body temperature did not affect male garter snakes'

(*Thamnophis sirtalis parietalis*) success in courting or obtaining mates and that matings could occur at body temperatures of lower than 10°C. However, results from this study suggest that even if males of all sizes and thus body temperatures obtain matings, there may be large differences in the ability of their sperm to reach sperm storage sites within the female, affecting their eventual reproductive success.

Conclusions

Temperature has a large effect on both the composition and motility of sperm in an ejaculate in *Hoplodactylus maculatus*. Geckos maintained at higher temperatures produced a greater proportion of samples containing long, fertilising sperm. In addition, both the proportion of sperm motile and the tail beat frequency of sperm increased at higher temperatures. There was, however, a trade-off between swimming speed and the longevity of sperm, suggesting that sperm have a finite energy source. The temperature-dependence of sperm motility has important implications for nocturnal lizards that are unable to regulate their temperatures as precisely as diurnal species. Since body size affects the thermal profile of lizards, male size is also likely to have a large influence on sperm competition success.

Chapter 8: General Discussion

Mating systems and sperm competition influence both male and female reproductive characteristics across a wide range of taxa (e.g. mammals [Dixson, 1987; Harcourt and Gardiner, 1994; Heske and Ostfeld, 1990], birds [Briskie, 1993; Briskie and Montgomerie, 1992, 1993; Coker et al., 2002; McCracken, 2000] and insects [Gage, 1994; Parker, 1970]; also see Birkhead and Møller (1998) for reviews). However, to date comparative studies have largely overlooked the evolution of these traits in reptiles, despite this group having a high potential for sperm competition (Devine, 1984; Olsson and Madsen, 1998). Therefore, the primary aim of my research was to investigate whether mating system has influenced the evolution of reproductive traits in reptiles.

I found that there was more than a 40-fold variation in relative testis volume across species of lizards and snakes. This variation could not be explained by interspecific differences in life-history traits, such as seasonality of breeding, sex ratio or clutch size. This is supported by the finding that species of New Zealand geckos, which are very closely related and thus share many characteristics such as clutch size and seasonality of breeding, also have great interspecific variation in relative testis size. Therefore, it is most likely that this variation reflects differences in mating systems, as found in other taxa (Gage, 1994; Harcourt et al., 1995; Kusano et al., 1991; Møller, 1991; Stockley et al., 1997).

The mating system of lizards and snakes ranges from monogamy to polygynandry or promiscuity. Monogamy is extremely rare (Bull, 2000), and although it was once believed that the majority of lizards were polygynous (Ruby, 1981; Stamps, 1983), recent information suggests that most species are in fact polygynandrous (Devine, 1984; Olsson and Madsen, 1998). The lack of sexual dimorphism in head size among New Zealand geckos indicates that members of both sexes are subject to approximately equal intensities of sexual selection. Consequently, these species are unlikely to have polygynous or polyandrous mating systems. Moreover, the observation that New Zealand geckos fall in the middle of the observed range of relative testis size for other reptiles, coupled with the rarity of monogamy in lizards, suggests that these species are also likely to be polygynandrous, and thus experience sperm competition. The finding that there is great variation between species in relative testis size means that the degree of

polygynandry and intensity of sperm competition is also likely to be highly variable between these species, making this a particularly suitable group for addressing questions about sperm competition and the evolution of reproductive traits.

Hemipenis morphology was relatively conservative across species of geckos in my study, with all species having bilobed organs covered with calyculi, which may function as scraping or suctional devices (Cope, 1894, 1895). There was little interspecific variation in the surface features of the intromittent organ and observed variation was not related to relative testis size. Thus, sperm competition does not appear to have resulted in the evolution of more elaborate intromittent organs in these species. This contrasts with findings for other taxa, where males of species with more intense sperm competition often evolve features such as spines or bristles on their intromittent organs, facilitating the removal of rival male sperm (Coker et al., 2002; Dixon, 1987; Gage, 1992; Harcourt and Gardiner, 1994). There was, however, a significant positive relationship between hemipenis size and relative testis size, suggesting that species with more intense sperm competition have evolved longer intromittent organs. A similar relationship has been observed in other taxa (Brownell and Ralls, 1986; Coker et al., 2002; Dixon, 1987; Miller et al., 1998). Males of these species are likely to be able to insert their hemipenes further into the female reproductive tract, which may enable them to either place their sperm more favourably (Eberhard, 1985) or access areas containing the sperm of rival males, facilitating their removal (Waage, 1979).

It has been suggested that sperm competition also influences the evolution of sperm and ejaculate characteristics. Males producing ejaculates containing a greater number of sperm are likely to have a competitive advantage in sperm competition (Gage and Barnard, 1996; Parker, 1982, 1993; Stockley et al., 1997). Moreover, in some taxa it has been shown that sperm size is related to swimming speed (Gomendio and Roldan, 1991) and that sperm competition also selects for the production of larger sperm (Balshine et al., 2001; Briskie et al., 1997; Dixon and Birkhead, 1997; Gage, 1994; Gomendio and Roldan, 1991; LaMunyon and Ward, 2002; Morrow and Gage, 2000; Pitnick and Markow, 1994). I investigated this latter relationship in New Zealand geckos. Although there was more than a two-fold difference in sperm length between species, there was no relationship between sperm length and relative testis size. A similar lack of relationship has been found in other studies (Briskie and Montgomerie, 1992; Gage

and Freckleton, 2003) and suggests that some other evolutionary force is driving this diversification in sperm size.

The majority of reptiles studied to date have the ability to store sperm in specialised sperm storage tubules (SSTs) (Birkhead and Møller, 1993; Girling, 2002; Gist and Jones, 1989; Olsson and Madsen, 1998; Sever and Hamlett, 2002). Similarly, all species of New Zealand geckos examined possessed putative SSTs throughout their oviducts. There was a large amount of interspecific variation in both the number and size of SSTs. This variation was not due to seasonal effects and could not be explained by variation in relative testis size and sperm competition intensity. However, the number of SSTs both in the uterus and in total was negatively correlated with sperm length. Thus, species in which females have fewer SSTs have had a concurrent increase in sperm length among males. A similar correlation was found in birds by Briskie and Montgomerie (1992), who suggested that this is possibly an adaptation to sperm competition: sperm competition intensity is likely to be higher in species with fewer SSTs; thus males have evolved longer sperm, enabling them to reach SSTs more quickly. Unexpectedly, interspecific variation in SST size was not related to sperm length, unlike in other taxa (Briskie and Montgomerie, 1992, 1993; Briskie et al., 1997; Pitnick and Markow, 1994; Pitnick et al., 1999; Presgraves et al., 1999). Since I could not detect whether sperm were present in SSTs, this lack of a relationship could be due to variation in activity of SSTs between individuals: active SSTs are often larger than inactive SSTs (Birkhead et al., 1997; Briskie, 1994, 1996). Alternatively, it could reflect the mechanism of sperm storage in this group. Whereas bird SSTs often accommodate the ejaculates of several males through sperm layering (Briskie, 1993, 1994, 1996), New Zealand gecko SSTs may contain only one layer of sperm; thus males may be able to block SSTs with their sperm and prevent access to other males.

Results from my cross-species comparisons show that not only do species of New Zealand geckos vary greatly in relative testis size, and thus the predicted intensity of sperm competition, but that several reproductive characteristics of both males and females have also been exposed to evolutionary pressures arising from sperm competition. Although such studies reveal a lot of information about evolutionary forces operating across species, single species studies are also important, both to determine the accuracy of predictions made from broad patterns and also to ask more specific questions.

Hoplodactylus maculatus is a highly gregarious species of gecko. Not only does this species occur at much greater densities in the field than other lizards (McIvor, 1972, and references therein), but I also observed multiple individuals inhabiting the same rock crevice, including same sex combinations. This finding was supported by observations of captive populations, where it was not unusual to find multiple males and females occupying the same shelter boxes. These associations were found throughout the year, including during the mating season. Although fighting did occur between individuals of both sexes, this never appeared to result in injury to either party and was also not dependent upon season. These observations suggest that this species is not territorial at any time of the year, which, combined with a lack of sexual dimorphism in head and body size, indicates that *H. maculatus* is unlikely to have a polygynous or polyandrous mating system.

H. maculatus had a peak courtship period from February to May and potentially a second season in early spring, from September to October. On average, each individual courted or was courted by three individuals of the opposite sex. There was no evidence of pair bonds forming in the captive populations and mate guarding was never seen. Consequently, it is highly unlikely that this species has a monogamous mating system. These findings suggest that *H. maculatus* has a polygynandrous mating system, where both males and females are promiscuous. This supports the predictions made from the cross-species comparison of relative testis size. *H. maculatus* from this region (*H. maculatus* “Canterbury”) lay in the middle of the range of relative testis size among New Zealand lizards. Thus, those species with larger testes are likely to have even more promiscuous mating systems, whereas those with smaller testes will experience a lower intensity of sperm competition.

I discovered that male *H. maculatus* produce two different types of sperm which have very discrete size distributions, the longer being more than twice the length of the shorter. ‘Long’ sperm were produced in much lower numbers than ‘short’ sperm, being present in only 30% of samples. However, there was a positive relationship between temperature and the proportion of samples containing long sperm. This may suggest that males need to experience higher temperatures for a longer duration to replenish long sperm than is necessary for short sperm, indicating that long sperm require a greater energy investment. Alternatively, if the long sperm are more energetically expensive to

produce, it is possible that males only ejaculate these at higher temperatures when they are more likely to be successful. Long sperm were often immotile and frequently observed with their heads bent back upon themselves. These observations may indicate that long sperm were not yet fully mature and that they may not complete maturation until they reach the female reproductive tract, as found in the lizard *Cnemidophorus sexlineatus* (Newton and Trauth, 1992). Although sperm of both morphs had the same tail beat frequencies, their swimming patterns also differed: long sperm swam unidirectionally whilst short sperm often swam in circles. Finally, the DNA content of these two morphs differed. Whereas, the long sperm contained DNA, the short sperm did not, meaning that they must serve some function other than direct fertilisation of the egg. Although it has been suggested that some mammals may produce different types of sperm serving different functions (Baker and Bellis, 1988, 1989; Bellis et al., 1990; Kura and Nakashima, 2000), evidence is still lacking (Harcourt, 1989, 1991). Thus sperm polymorphism has not previously been conclusively demonstrated in vertebrates. Moreover, although sperm polymorphism is relatively widespread in invertebrates, the production of sperm lacking DNA has only been found in Lepidoptera (Silberglie et al., 1984; Swallow and Wilkinson, 2002). Several theories about the function of these sperm have been proposed, including the facilitation of transport of fertilising sperm (Buckland-Nicks et al., 1982; Friedländer and Gitay, 1972; Holt and North, 1970; Oppliger et al., 2003) and serving as a nutrient source (Silberglie et al., 1984; Swallow and Wilkinson, 2002; Watanabe et al., 2000). However, the most convincing explanation to date is that these sperm have evolved in response to sperm competition. It has been proposed that non-fertilising sperm may aid in sperm competition either through blocking or disabling sperm of rival males or by filling sperm storage sites and thus preventing entry of other sperm (Silberglie et al., 1984).

An ecological variable that is likely to have a large effect on sperm viability and thus sperm competition success in reptiles is temperature as, unlike birds and mammals, reptiles are ectothermic and thus experience quite large fluctuations in body temperature. This is especially true for nocturnal species, which have little opportunity to regulate their body temperature during their peak activity period and thus often function at sub-optimal temperatures (Angilletta and Werner, 1998; Dial, 1978; Huey et al., 1989; Huey and Slatkin, 1976). Since *H. maculatus* is nocturnal and is expected to have a relatively

high intensity of sperm competition, it was an ideal subject to study the influence of temperature on sperm viability.

In *H. maculatus* there was a positive relationship between temperature and the proportion of motile sperm. Moreover, of those sperm that were motile, tail beat frequency also increased with temperature. A similar relationship has been found in externally fertilising fish (Billard and Cosson, 1992). There was, however, a concurrent decrease in motility through time at higher temperatures, suggesting that sperm have a finite energy source resulting in a trade-off between motility and longevity, as seen in other taxa (Billard and Cosson, 1992; Levitan, 2000; Vladic and Järvi, 1997; but see Gage et al., 2002). These findings suggest that males transferring sperm at higher body temperatures are likely to be more successful under sperm competition, as not only will a greater proportion of their sperm be motile but those sperm that are motile will also be able to reach sperm storage sites more rapidly. This finding has large implications for male lizards. For example, it suggests that males that differ in body size are also likely to differ in sperm competition success, as body size can have a large effect on temperature relations. The direction of this success will be largely dependent upon time of day: larger individuals cool less rapidly (Bartholomew, 1982; Hertz et al., 1993) and thus would be more successful earlier in the evening; however, smaller individuals are able to take advantage of smaller variations in microclimate (Cowles, 1941; Stevenson, 1985) potentially enabling them to mate further into the night, and will also be able to heat more rapidly in the morning. Since *H. maculatus* is active all night and appears to mate throughout the 24-hour period, this could be a successful strategy for these smaller males which were found to be less successful in obtaining courtships than their larger counterparts.

Future Research

This study has investigated the relationship between sperm competition and reproductive characteristics in New Zealand geckos using many evolutionary questions previously formulated for other taxa. However, there are still many areas that have not been explored and my findings have given rise to many more questions.

To obtain a better understanding of the relationship between mating system and relative testis size in reptiles, a much greater amount of information is required about

the mating system of more species. Detailed information is currently available for only a few species, and these studies have shown that there is often great disparity between the social mating system of a species and the genetic mating system (Bull, 2000). Thus, future researchers investigating the mating systems of species are encouraged to combine both behavioural and genetic data. Similarly, since only the social mating system of *Hoplodactylus maculatus* was determined in this study, genetic techniques should be used in the future to determine the paternity of offspring. This will enable us to deduce whether females are producing mixed broods and also to relate male traits such as hemipenis size and sperm length to mating success of males.

Future research should also address the hypothesised interaction between body size, temperature and sperm competition success. This will allow us to determine whether male body size affects reproductive strategies, such as the timing of copulation with respect to environmental temperature. It would also be interesting to investigate whether body size and temperature affect the proportion of sperm reaching sperm storage sites and their ultimate fertilisation success.

Perhaps the most exciting finding from this research is that *H. maculatus* males produce two sperm morphs, one of which does not contain DNA. This raises many possible future research questions and directions. Firstly, it should be determined how widespread this phenomenon is amongst lizards. Although I did not find multiple sperm morphs in any other species of gecko studied, either from fresh ejaculates or through the dissection of epididymides, this needs to be investigated more thoroughly as sample sizes were very small in all cases. In addition, the exact processes involved in the production of each morph should be determined, i.e. how and where the two types of sperm are produced in the testes. It would also be interesting to investigate the proportional representation of sperm from each morph in a natural ejaculate and the fate of these sperm in the female reproductive tract, e.g. do both morphs reach sperm storage sites or do short sperm remain in the vaginal region? Finally, the role of the non-fertilising sperm in fertilisation needs to be determined.

My research highlights the fact that we still know very little about lizard reproductive systems despite this group being particularly suited to studies of this nature. This is especially true for the rich array of lizard species in New Zealand. Reptiles appear to have a high potential for sperm competition. Moreover, in general they are much easier

to manipulate than many bird and mammalian species and, due to their ectothermy, raise many interesting questions in terms of relations between temperature and reproductive physiology. Undoubtedly research into this group will yield many more exciting findings in the future.

Acknowledgements

I would like to thank Dr Jim Briskie for his supervision of this work, the provision of equipment and facilities and for his encouragement throughout. Thanks also to the other members of my supervisory committee, Assoc. Prof. Colin McLay and Dr Bruce Waldman, for their helpful suggestions and the provision of equipment, and to Dr Alison Cree and Assoc. Prof. Mats Olsson for constructive criticisms of the thesis.

I am very grateful to the Foundation for Research, Science and Technology, the University of Canterbury, the Shirtcliffe Fellowship and the Brian Mason Trust for providing scholarships and funding for this research. In addition, this research would not have been possible without the technical support provided by the School of Biological Sciences. In particular, I would like to thank Jan McKenzie, who provided help with light and scanning electron microscopy and working with digital images, Neil Andrews, for assistance with scanning electron microscopy, Graeme Bull, for his help with the histological preparation of samples, Manfred Ingerfeld, for instruction in the use of Image-Pro Plus, and Jandouwe Villinger, for help with EthoVision Color-Pro. I am also very grateful to Nick Etheridge and Victor Mencil, who helped to construct the enclosures, Renny Bishop, who introduced me to some collection sites and techniques at the beginning of this research, and Linda Morris for the supply of *Drosophila*. Thanks also to Robert Ewers for production of the field site locality map and Dr Dru Mason for the provision of Dulbecco's Modified Eagle's Medium. Special thanks go to Dr Ashley Sparrow and Dr Raphael Didham for their help with statistical procedures and their immense patience and enthusiasm. Thanks also to Dr Bob Montgomerie and his research group at Queen's University, Kingston, for introducing me to the various sperm motility packages and for advice on sperm collection and storage.

The Museum of New Zealand Te Papa Tongarewa and Canterbury Museum provided the majority of preserved specimens used in this study. Many thanks to Raymond Coory and Dr Paul Scofield for providing permission and facilities for this work. Thanks also to Tony Whitaker, for his helpful comments and suggestions and for allowing me to analyse his collection of *Hoplodactylus maculatus*, and to Bruce Thomas, Landcare Research, for providing access to these specimens and for sharing his enthusiasm for lizards. Many thanks to Ivan Borich, Ti Point Reptile Park, for allowing me to collect sperm samples from his captive lizards.

Many people were consulted during this study. In particular, I would like to thank Tony Whitaker, Dr Alison Cree, Peter Gaze, Bernard Goetz, Dr Brian Gill, Rod Rowlands, Dr Rod Hitchmough, David Scheltinga, Prof. Jeff Duckett, Warwick Brown and members of the New Zealand Herpetological Society, for providing invaluable information, especially whilst the project was in its infancy.

Permits for this work were provided by the Department of Conservation. In particular, I would like to thank Euan Kennedy, Andy Grant, Kylee Simpson, Peter Gaze, Robin Smith, Dr Mandy Tocher and Kerry Weston for the issuing of permits and provision of information concerning the distribution of geckos in the South Island. I would also like to thank Te Runanga o Ngai Tahu for providing their permission to study gecko populations on Banks Peninsula. Thanks also to Pat and Paul Pritchett, for assistance with the provision of materials and construction of the enclosures, and for allowing me to keep the enclosures on the back lawn. I am also very grateful to the many landowners who provided permission for me to access and collect geckos from their properties. In particular I would like to thank Hugh Wilson, Hinewai Reserve, for providing distribution maps of *Naultinus gemmeus* and for introducing me to his population of *H. maculatus* in the power box outside his house. Thanks also to Reg MacIntosh (Devil's Knob), Daniel and Joan Barrar (Gebbies Pass) and the owners and managers of Peraki Farm (Devil's Gap) and Kinloch Station (Te Oka Peak), for allowing access to their properties. In addition, I am very grateful to the Christchurch City Council for allowing me to collect geckos from reserves around the Port Hills.

This research would not have been possible without the help of field assistants. I would like to thank Eric Todd, Jan Todd, Stephen Jennings and Kathryn Atkinson for their assistance in the field. I am especially grateful to Matt Todd for his long-term commitment to this project. Matt not only provided immense support and encouragement throughout this research but also watched geckos on cold nights and was a constant companion in the field. Without his immense knowledge of Banks Peninsula, excellent gecko finding skills and mountain-goat characteristics whilst working on rock outcrops this research would have been next to impossible and far less enjoyable. Finally, I would like to thank the many geckos who participated in this study.

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Appendix A

Species of New Zealand gecko used in cross-species comparison of testis size and sexual size dimorphism. Collection sites for all individuals used in the study are listed (M = male, F = female) and inferred times of mating and ovulation are presented. Museum at which specimens are held: T = Museum of New Zealand Te Papa Tongarewa, C = Canterbury Museum.

Species	Mean SVL (mm)	Mean testis vol (mm ³)	Collection site	Museum	Time of mating	Time of ovulation
<i>Hoplodactylus chrysosireticus</i> [‡]	60.30	25.69	Mana Island (1M)	T	April ^{1,2} ; Autumn? [‡]	Sept-Nov? [‡]
<i>H. duvaucelii</i>	116.38	116.39	Middle Island (3M, 1F)	T	Sept-Oct ² ; Spring? [‡]	Sept-Nov? [‡]
			Korapuki Island (1M)	T		
			Green Island (2M)	T		
			Coppermine Island (2M, 3F)	T		
			Whatupuke Island (1M [□] , 1M, 2F)	T		
			Bream Island (1F)	T		
			Sentinel Rock (1F)	T		
			The Brothers (1M)	T		
<i>H. granulatus</i>	75.33	54.64	Hikurangi (1F)	T	April-May and Sept-Oct ² ; Autumn? [‡]	Sept-Nov? [‡]
			Rangataua (1M, 2F)	T		
			Plimmerton (1M)	T		
			Trentham (1M)	T		

Species	Mean SVL (mm)	Mean testis vol (mm ³)	Collection site	Museum	Time of mating	Time of ovulation
			Pinehaven (1F)	T		
			Whiteman's Valley (1F)	T		
			Ngaio (1M)	T		
			Khandallah (1F)	T		
			Wellington (1M ^Y)	C		
			Days Bay (1F)	T		
			Unknown (4M, 5F)	T		
<i>H. maculatus</i>	63.45	12.24	Ngatapa (1M)	T	Feb-May ^{1,2,3}	Sept-Oct ³
			NW Masterton (1M)	T		
			W of Mikimiki (1M, 4F)	T		
			Tawaha (2F)	T		
			Palliser Bay (2F)	T		
			Cape Palliser Lighthouse (1F)	T		
			Stephens Island (3M, 2F)	T		
			Cable Bay (2M, 1F)	T		
"Canterbury" [§]	60.53	17.17	Motunau Island (3M, 3F)	T	Feb-May ⁴ ; Autumn? [‡]	late Oct ⁴ ; Sept-Nov? [‡]
			Port Hills (2F)	T		
			Allandale (1M)	T		
			Birdlings Flat (4M, 6F)	T		

Species	Mean SVL (mm)	Mean testis vol (mm ³)	Collection site	Museum	Time of mating	Time of ovulation
“Eastern Otago” §	66.37	16.21	1/2 mile above Clyde (1M, 1F)	T	Feb-May ⁵ ; Autumn? [‡]	Sept ⁵ ; Sept-Nov? [‡]
			Clarkes Junction (1M, 1F)	T		
			Otago (1M [†])	T		
			Longridge (2M, 1F)	T		
			Conical Hill (2M, 2F)	T		
“Marlborough” §	49.69	9.39	Ohio Bay, Wellington (2M, 1F)	T	Feb-May? [*] ; Autumn? [‡]	Sept-Nov? [‡]
			White Bluffs (2M, 2F)	T		
			Haldon Hills, Marlborough (1F)	T		
			Waipapa Bay (4M, 3F)	T		
			Marlborough (2M, 4F)	T		
			Horseshoe Lake (1M, 1F)	T		
			Waiau River (2M)	T		
			Hope River (1M)	T		
			Godley River (1F)	T		
“Poor Knights” §	74.45	43.87	Archway Island (4M, 1F)	T	Feb-May? [*] ; Autumn? [‡]	Sept-Nov? [‡]
			Aorangaia Island (1M, 3F)	T		
			Stack between Tawhiti Rahi Island and Aorangi Island (1M, 2F)	T		
			Tawhiti Rahi Island (2M, 3F)	T		

Species	Mean SVL (mm)	Mean testis vol (mm ³)	Collection site	Museum	Time of mating	Time of ovulation
“Southern Alps” §	61.72	12.16	Clarence River (1M)	T	Feb-May? [*] ; Autumn? [‡]	Sept-Nov? [‡]
			Charwell (1F)	T		
			Waiau River (2M, 3F)	T		
			Hope River (1M)	T		
			Amo, Waimate (1F)	T		
			Burkes Pass (1M, 5F)	T		
			Islands in Lake Tekapo (1F)	T		
			Archerson River (1F)	T		
			Mt Taranoka (1F)	T		
			Mt Harris (1M, 1F)	T		
“Three Kings” §	81.54	14.63	NE Island (2M, 2F)	T	Feb-May? [*] ; Autumn? [‡]	Sept-Nov? [‡]
			Great Island (3M, 1F)	T		
			Tooman Bay, Great Island (1F)	T		
			SW Island (2F)	T		
<i>H. pacificus</i>	72.47	22.44	Mt Camel, Houhora (1F)	T	March-May ^{1,2} ; Autumn? [‡]	Sept-Nov? [‡]
			Motukamara Island (1M)	T		
			Coppermine Island (1F)	T		
			Sail Rock (5M)	T		
			Little Ohena Island (1M, 1F)	T		

Species	Mean SVL (mm)	Mean testis vol (mm ³)	Collection site	Museum	Time of mating	Time of ovulation
<i>Naultinus elegans elegans</i>	64.30	30.42	Middle Island (7M, 1F)	T	Sept/Nov ² ; Spring? [‡]	Nov-Dec? [‡]
			Half Island (1F)	T		
			Karewa Island (1M)	T		
			Onetahi, Whangarei (1F)	T		
			Okahukura Peninsula (1M)	T		
			Conical Hill, E of Middlemore (1M)	T		
			Auckland District (1M)	T		
			Harapepe / Te Pahu (1M)	T		
			Oparau (1M)	T		
			Captive Bred (4F)	T		
<i>N. e. punctatus</i>	72.91	37.06	Wharerata, SE Mahia (1F)	T	July-Aug ² ; Spring? [‡]	Nov-Dec? [‡]
			Palmerston North (1F)	T		
			Plimmerton (1F)	T		
			Naenae (1M)	T		
			Stokes Valley (2F)	T		
			Whitemans Valley (2M)	T		
			Lower Hutt (1F)	T		
			Petone (1M)	T		
			Days Bay, Wellington (1M, 1F)	T		

Species	Mean SVL (mm)	Mean testis vol (mm ³)	Collection site	Museum	Time of mating	Time of ovulation
			Catchpod Stream, Wainuiomata (1M [†])	T		
			York Bay, Wellington (1M)	T		
			Butterfly Creek, Eastbourne (1F)	T		
			Wellington District (1M)	T		
			Captive Bred (1F)	T		
<i>N. gemmeus</i>	65.20	22.90	Allandale (1M)	T	Sept-Oct ^{1,2} ; Spring? [‡]	Nov-Dec? [‡]
			Diamond Harbour (1F)	T		
			Purau Valley (1M, 1F)	T		
			Akaroa (1M [¥])	C		
			Portobello (1M, 1F)	T		
			Otago Peninsula (1M, 2F)	T		
<i>N. grayii</i> [¥]	70.20	12.40	Awanui (1M)	T	Aug-Sept ^{1,2} ; Spring? [‡]	Nov-Dec? [‡]
			Captive Bred (1M)	T		
<i>N. manukanus</i>	60.48	23.43	Stephens Island (1M, 1F)	T	June-Oct ^{1,2} ; Spring? [‡]	Nov-Dec? [‡]
			Bay of Many Coves, Queen Charlotte Sound (1M, 1F)	T		
			Otanerau, Queen Charlotte Sound (1F)	T		
			Picton (1M [□])	T		

Species	Mean SVL (mm)	Mean testis vol (mm ³)	Collection site	Museum	Time of mating	Time of ovulation
			Erie Bay, Marlborough (1M [□])	T		
			Captive (2F)	T		
<i>N. rakiurae</i> [¥]	57.20	11.37	Stewart Island? (1M)	T	Spring? [‡]	Nov-Dec? [‡]
<i>N. rudis</i> [¥]	62.56	21.29	Mt Fyffe, Kaikoura (1M)	T	June/Oct ² ; Spring? [‡]	Nov-Dec? [‡]
			Hanmer Springs (1M)	T		
			Hanmer Springs (1M)	C		
<i>N. stellatus</i> [¥]	62.50	16.37	Collingwood (1M)	C	Aug-Oct ^{2,6} ; Spring? [‡]	Nov-Dec? [‡]
			St Arnaud (1M)	C		
			Lake Rotoiti (1M)	C		

Notes

[§] Members of the *Hoplodactylus maculatus* species complex

[†] Specimens not included in testis size analysis due to missing testes; [¥] Species / specimens not included in sexual size dimorphism analysis; [□] Head dimensions not recorded due to specimen damage

* Mating season assumed to be the same as other members of *H. maculatus* species complex

References

¹ Gill and Whitaker, 1996; ² Rowlands, 1981; ³ Robinson, 1982; ⁴ McIvor, 1972; ⁵ MacAvoy, 1973; ⁶ Mainwaring, 1979

^{*} Proposed by Robinson, 1982

Appendix B

Species of overseas lizards and snakes used in cross-species comparison of testis size. Mean testis volume: original and converted data presented; State: P = preserved, Fr = fresh; Seasonal: Y = seasonal, N = non-seasonal; Sex ratio: M = male-biased, F = female-biased, 1:1 = even.

Family	Species	Mean SVL (mm)	Mean testis vol (mm ³)	State	Mean clutch size	Seasonal	Sex ratio	Mating system	References
Sauria									
Agamidae:									
Agaminae	<i>Agama agama africana</i>	94.00	52.43			N			Daniel, 1960
	<i>Agama cyanogaster</i>	102.00	78.12		11.0	Y			Robertson et al., 1965
	<i>Amphibolurus adelaidensis</i>	50.00 [†]	41.89		2.0	Y			Davidge, 1980; [†] Cogger, 1975
	<i>Amphibolurus isolepis</i>	70.00	18.91	P	3.3	Y			Pianka, 1971
	<i>Amphibolurus minor</i>	160.00 [†]	508.94		6.0	Y			Davidge, 1980; [†] Cogger, 1975
	<i>Draco quinquefasciatus</i>	96.01	20.94	P	3.0	N			Inger and Greenberg, 1966
	<i>D. melanopogon</i>	79.78	10.30	P	2.0	N			Inger and Greenberg, 1966
Leiolepidinae	<i>Uromastix hardwicki</i>	177.00	342.84	Fr		Y			Arslan et al., 1978
Iguanidae:									
Crotaphytinae	<i>Gambelia silus</i>	106.10	48.67		2.9	Y			Tollestrup, 1982
	<i>G. wislizenii</i>	104.60	39.79		5.6	Y			Tollestrup, 1982
Phrynosomatinae	<i>Sceloporus horridus</i>	83.20	10.39	P	14.8	Y			Valdéz-González and Ramírez-Bautista, 2002
	<i>S. malachiticus</i>	75.00	59.63	P	5.9	Y			Marion and Sexton, 1971
	<i>S. mucronatus</i>	83.75	175.00	P	5.1	Y			Méndez de la Cruz et al., 1988
	<i>S. occidentalis</i>	70.00	132.48			Y			Wilhoft and Quay, 1961

Family	Species	Mean SVL (mm)	Mean testis vol (mm ³)	State	Mean clutch size	Seasonal	Sex ratio	Mating system	References
Polychrotinae	<i>S. orcutti</i>	102.00	225.00	Fr	11.0	Y			Mayhew, 1963
	<i>S. spinosus</i>	90.40	14.50	P	18.5	Y			Valdéz-González and Ramírez-Bautista, 2002
	<i>S. woodi</i>	47.60	55.69	P	4.1	Y			Jackson and Telford, 1974
	<i>Uma inornata</i>	115.00 [†]	176.70			Y			Mayhew and Wright, 1970; [†] Smith, 1995
	<i>U. notata</i>	96.00	123.30	Fr	2.0	Y			Mayhew, 1966
	<i>Anolis auratus</i>	43.50	25.95	Fr	1.0	Y			Sexton et al., 1971
	<i>A. carolinensis</i>	65.00	37.16	Fr		Y		Polygynous [‡]	Fox, 1958; [‡] Jenssen and Nunez, 1998; Jenssen et al., 2000
	<i>A. conspersus</i>	63.50	23.15	P	1.0				Licht and Gorman, 1970
	<i>A. cybotes</i>	66.70	55.96	P	1.0				Licht and Gorman, 1970
	<i>A. grahami</i>	63.90	43.58	P	1.0	Y			Licht and Gorman, 1970
	<i>A. limifrons</i>	44.20	24.78	Fr	1.0	Y			Sexton et al., 1971
	<i>A. lineatopus</i>	62.50	39.65	P	1.0	Y		Polyandrous [‡]	Licht and Gorman, 1970; [‡] Olsson and Madsen, 1998 (and references therein)
	<i>A. oculatus</i>	54.90	11.50	P	1.1	N	M		Somma and Brooks, 1976
	<i>A. opalinus</i>	48.80	31.63	P	1.0	N			Jenssen and Nunez, 1994
	<i>A. richardi griseus</i>	93.20	71.16	P	1.0	N			Licht and Gorman, 1970
	<i>A. sagrei</i>	46.50	42.20	P	1.0	Y		Polygynous [‡]	Licht and Gorman, 1970; [‡] Schoener and Schoener, 1980
	<i>A. trinitatis</i>	63.30	26.28	P	1.0	N			Licht and Gorman, 1970

Family	Species	Mean SVL (mm)	Mean testis vol (mm ³)	State	Mean clutch size	Seasonal	Sex ratio	Mating system	References
	<i>A. tropidogaster</i>	44.90	31.24	Fr	1.0	Y			Sexton et al., 1971
Gekkonidae:									
Gekkoninae	<i>Cyrtodactylus pubisculus</i>	66.35	28.13	P	2.0	N			Inger and Greenberg, 1966
	<i>C. malayanus</i>	98.69	49.83	P	2.0	N			Inger and Greenberg, 1966
	<i>Gonatodes albogularis</i>	39.00	12.53	P	1.0	N			Sexton and Turner, 1971
	<i>Hemidactylus turcicus</i>	49.10	29.03	P		Y	1:1		Meshake, 1995
Pygopodinae	<i>Delma fraseri</i>	120.00 [†]	125.66		2.0	Y			Davidge, 1980; [†] Wilson and Knowles, 1988
	<i>Pletholax gracilis</i>	75.00 [†]	8.38		2.0	Y	F		Davidge, 1980; [†] Wilson and Knowles, 1988
	<i>Pygopus lepidopodus</i>	230.00 [†]	94.25		2.0	Y	F		Davidge, 1980; [†] Wilson and Knowles, 1988
Lacertidae:	<i>Takydromus hsuehshanensis</i>	60.50	42.33	Fr	3.2	Y			Huang, 1998
Cordylidae:									
Cordylinae	<i>Pseudocordylus m. melanotus</i>	118.30	250.00			Y			Flemming, 1993
Scincidae:									
Lygosominae	<i>Cryptoblepharus plagiocephalus</i>	45.00 [†]	32.99		2.0	Y			Davidge, 1980; [†] Wilson and Knowles, 1988
	<i>Ctenotus brooksi</i>	44.40	15.20	P	2.2	Y			James, 1991
	<i>Ctenotus helenae</i>	76.70	56.00	P	4.3	Y	M		James, 1991
	<i>Ctenotus leonhardii</i>	65.90	22.00	P	3.3	Y			James, 1991
	<i>Ctenotus lesueurii</i>	100.00 [†]	130.90			Y	M		Davidge, 1980; [†] Wilson and Knowles, 1988
	<i>Ctenotus pantherinus</i>	79.80	95.00	P	6.6	Y	F		James, 1991

Family	Species	Mean SVL (mm)	Mean testis vol (mm ³)	State	Mean clutch size	Seasonal	Sex ratio	Mating system	References
	<i>Ctenotus piankai</i>	48.60	16.00	P	2.8	Y	M		James, 1991
	<i>Ctenotus quattuordecimlineatus</i>	57.60	33.80	P	3.1	Y	M		James, 1991
	<i>Ctenotus schomburgkii</i>	42.10	10.10	P	2.1	Y	M		James, 1991
	<i>Egernia whitii</i>	97.50	65.03	Fr	2.9	Y	F		Milton, 1987
	<i>E. modesta</i>	97.50	52.03	Fr	2.7	Y	F		Milton, 1987
	<i>Lampropholis guichenoti</i>	35.95	16.30	Fr	2.3	Y	1:1		Simbotwe, 1985
	<i>Leiopisma fuscum</i>	62.00	42.93	P	2.0	Y			Wilhoft and Reiter, 1965
	<i>Lerista elegans</i>	40.00 [†]	8.38		2.0	Y	M		Davidge, 1980; [†] Wilson and Knowles, 1988
	<i>Lerista lineata</i>	55.00 [†]	12.57		3.0	Y	M		Davidge, 1980; [†] Wilson and Knowles, 1988
	<i>Mabuya striata striata</i>	75.50	47.55		7.0	N			Robertson et al. 1965
	<i>Niveoscincus ocellatus</i>	60.00	107.40	Fr	2.7	Y			Jones et al., 1997
	<i>Oligosoma suteri</i>	79.00	49.44	Fr	3.7	Y			Towns, 1975
	<i>Tiliqua scincoides scincoides</i>	284.38	1154.31	P		Y			Shea, 1993
Serpentes									
Viperidae:									
Viperinae	<i>Bitis arietans arietans</i>	1400.00 [†]	3685.23		42.0	Y			Robertson et al., 1962; [†] Zug et al., 2001
Colubridae:									
Colubrinae	<i>Psammophis sibilans sibilans</i>	1059.00	2293.47						Robertson et al., 1962
Elapidae:									
Hydrophiinae	<i>Austrelaps superbus</i>	76.60	369.30	Fr	16.5 [□]	N			Shine, 1977a; [□] Shine, 1977b